201-14835

November 6, 2003

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The Honorable Michael O. Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know
HPV CONSORTIUM
Gas Oils Test Plan

Dear Administrator Leavitt:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Gas Oils Test Plan and Robust Summary. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and the associated test plans. We are therefore submitting this test plan, as well as the robust summary, directly to EPA to make available for public comment.

Electronic copies of the test plan (in .pdf format) and robust summary (in .pdf format and as an IUCLID export file) are accompanying this letter via email to the EPA HPV robust summary email address (http://www.epa.qov/chemrtk/srbstsum.htm). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdokl@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee and Technical Workgroup

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

GAS OILS CATEGORY

Submitted to the US EPA

by

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration

November 3, 2003

Table of Contents

Plain Language Summary

The gas oils category includes both finished products (distillate fuels) and the refinery streams (gas oils) from which they are blended. The materials in this category, together with those in the Jet Fuel/Kerosene HPV category, constitute a generic class of petroleum substances commonly known as middle distillates. The distillate fuels covered in this category are used in diesel engines and for both industrial and domestic heating. While within the refinery the gas oil streams exist primarily as intermediates in closed systems. Selected gas oil streams may ultimately be blended into distillate fuels, marine bunker fuels and occasionally into lubricants. At ambient temperatures, all the substances in this category are liquids. Gas oil streams and distillate fuels are complex petroleum mixtures, composed primarily of saturated (paraffinic and naphthenic) or aromatic hydrocarbons with carbon numbers ranging from C_9 to C_{30}. The basic strategy of this test plan for characterizing the human health and environmental hazards of category members is to use data from gas oil streams that are higher in one of these two chemical classes to demonstrate the boundary of toxicity for materials in this test plan and to predict the potential hazards of untested gas oil streams.

The substances included in the Gas Oils category are similar from both a process and physical-chemical perspective, being differentiated from each other primarily by their aromatic and saturated hydrocarbon content. The compositions of the gas oil streams range from those that are predominantly saturated hydrocarbons to those that are predominantly aromatic hydrocarbons. Consequently, the category can be considered a continuum, bounded by materials that are compositionally either high in saturated hydrocarbons or aromatic hydrocarbons. While the ratio of the saturated and aromatic hydrocarbons may vary between category members the saturated and aromatic hydrocarbons species that make up the category members are similar. Based on the available data, the physical-chemical properties of an individual category member depend on its compositional makeup, vis a vis saturated and aromatic hydrocarbons. Therefore, gas oil streams that are predominantly saturated hydrocarbons will have similar physical-chemical properties, while those that are composed predominantly of aromatic hydrocarbons will have somewhat different properties. As products that are blended from the gas oil streams, the compositions of the distillate fuels fall within the range of the compositions shown by the gas oil streams and reflect the characteristics of the gas oils streams from which they are blended. Thus, the properties of representative samples of a blended distillate fuel and gas oils composed predominantly of saturated hydrocarbons or aromatic hydrocarbons can be used to demonstrate the boundary of toxicity for materials in this test plan and to understand the physical-chemical properties and toxicity of similar substances within the category.

A substantial body of data has been compiled on representative gas oil streams and distillate fuels. However, a thorough review of the existing data identified a lack of reproductive toxicity data on the materials in this category, and suggested the need for additional reproductive toxicity testing. Therefore, the Testing Group is proposing to perform reproductive/developmental screening studies (OECD 421) on two representative gas oil samples; one with a relatively high saturated hydrocarbons content and one with a relatively high aromatic hydrocarbons content. A reproductive/developmental screening study (OECD 421) will also be performed on a representative sample of a No. 2 distillate fuel, the substance in this category with the greatest potential for public exposure. As the data in the Robust Summary illustrate, distillate fuels and gas oil streams high in either saturated or aromatic hydrocarbons have similar effects with regard to several of the Screening Information Data Set (SIDS) mammalian toxicity endpoints, i.e. acute and repeat-dose mammalian toxicity. However, limited published literature indicate a gas oil's developmental toxicity, mutagenicity, and carcinogenicity correlate with the oil's aromatic content, specifically its 3-7 ring polycyclic aromatic compounds (PAC) content (Feuston, 1994). The Testing Group believes it is plausible that the gas oils reproductive toxicity also correlates with the PAC content.

Conducting these three studies will allow the Testing Group to:

- Complete the SIDS characterization of the mammalian toxicity on materials that represent the boundaries of the range of compositions found within the gas oils category, and
- Test the hypothesis that the reproductive toxicity of a gas oil stream correlates to its 3-7 ring PAC content.

The environmental fate characteristics of both gas oils and distillate fuels are due in large part to the physico-chemical parameters of the individual component hydrocarbons. Where physico-chemical data did not exist or were impractical to obtain, calculated physico-chemical and environmental data for selected constituents of gas

oils have been developed using the EPIWIN© computer model. Because biodegradation of gas oils has not been extensively studied, biodegradability testing of selected gas oils is proposed.

While the aquatic toxicity of distillate fuels has been adequately characterized, no data exist on the toxicity of individual gas oil streams. Therefore, the Testing Group proposes to test two gas oil streams, one with a relatively high saturated hydrocarbons content and one with a relatively high aromatic hydrocarbons content. Candidate streams also will be evaluated for their potential to produce the greatest water soluble (hence bioavailable) fraction in aqueous solutions (i.e., low carbon number).

The currently available data and that which will be generated by the proposed testing outlined in Table 2, combined with chemical characterization will provide sufficient information to predict the health and environmental hazards of the materials in the gas oils category.

Description of the Gas Oils Category

The Gas Oils category includes both finished products (distillate fuels) and the refinery streams (gas oils) from which they are blended. The specific CAS numbers and descriptions of category members are detailed in Appendix A. The nomenclature used to describe the distillate fuels (finished products) can be confusing. Table 1 shows synonyms that can be applied to various fuel types.

Table 1. Distillate Fuel Synonyms

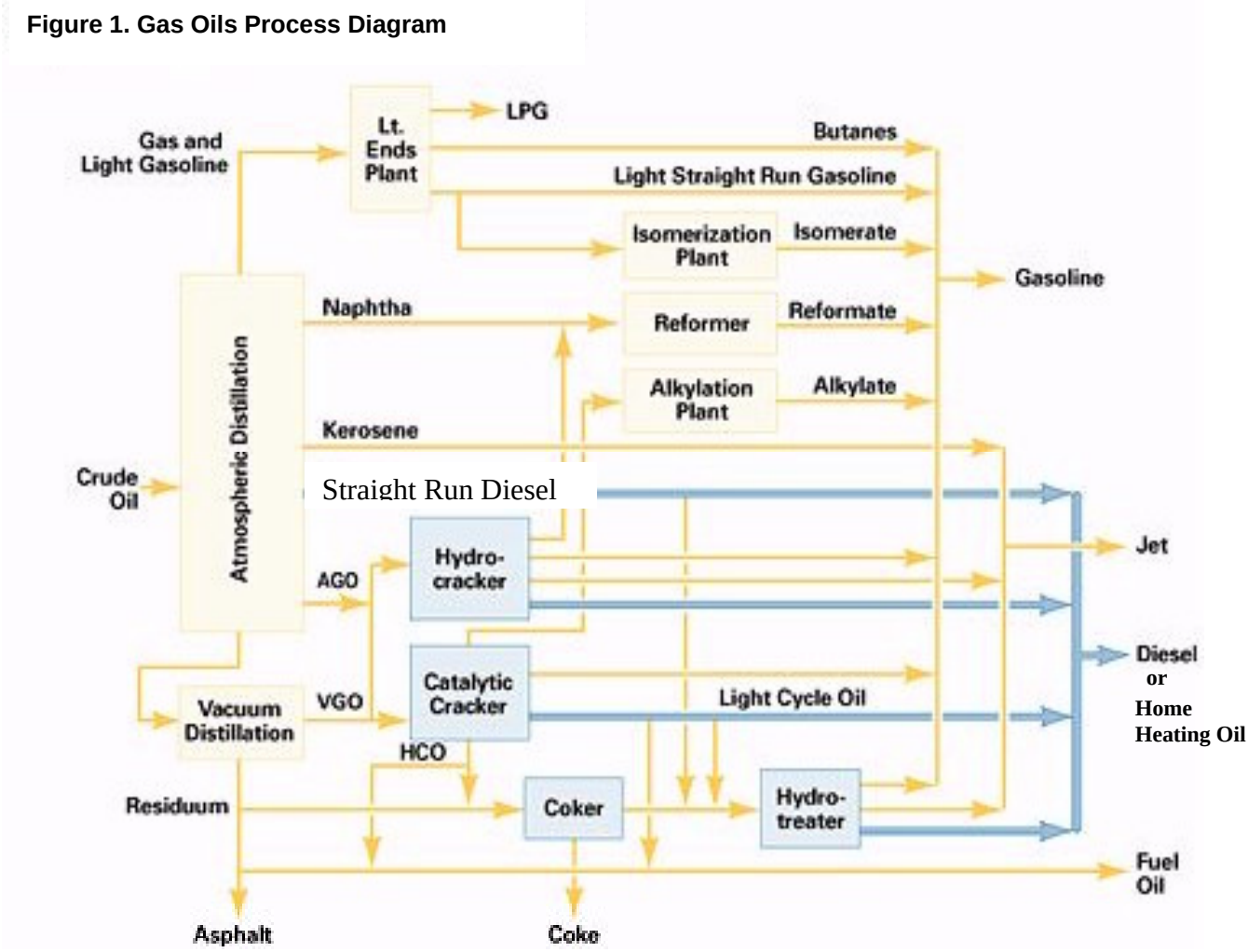
	Fuel Oil No. 1-D	Fuel Oil No. 2	Fuel Oil No. 2-D	Fuel Oil No. 4
Synonym(s)	Diesel fuel; Diesel fuel oil no. 1 Diesel oil no. 1 No. 1 diesel Diesel oil (light) Arctic diesel	API no. 2 fuel oil Gas oil Home heating oil no. 2 No. 2 burner oil Diesel fuel Furnace oil no. 2	Diesel fuel Diesel fuel oil no. 2 Diesel oil no. 2 No. 2 diesel Diesel oil (medium)	Oil, fuel no. 4 Residual fuel oil no. 4 No. 4 fuel oil Residual fuel oil Marine boiler fuel Marine diesel fuel Diesel fuel no. 4 Grade 4

ATSDR, 1995

The fuel oils covered in this category are used primarily as heating oils and as fuels in many types of internal-combustion engines. Fuel oils No. 1-D and No. 2-D have been used for automotive diesel engines, while No. 4 diesel fuel is used for low and medium speed diesel engines in non-automotive applications. Diesel fuel #2 is almost identical in chemical composition to Fuel Oil #2, with the exception of the additives. Fuel oil #2 has been used as a home heating oil and as an industrial heating oil. Fuel Oil #4 has been used in commercial and industrial burners to generate steam, for space and water heating, pipeline pumping, and gas compression (ASTM, 2001; 2002). Two other classes of fuel oils, Fuel Oil #1(also known as kerosene) and Fuel Oil #6 (heavy fuel oil) are covered in separate API HPV Test Plans.

As shown in Figure 1, gas oil streams are produced either as distillates of atmospheric distillation or by secondary processing of the materials derived from the vacuum distillation of the residuum from the atmospheric distillation of crude oil. Materials from this secondary processing have higher aromatic and olefin contents than straight run gas oils. Distillate fractions that require only minor or no additional processing are known as “straight run” gas oils. The distillate fuels may be straight run or a blend of various gas oil streams (both straight run and cracked). Historically, straight-run gas oils are the major components of the distillate fuels, but rising demand has made it necessary to use increasing volumes of streams derived from the secondary processing of heavier fractions. Because they are manufactured to meet performance specification limits (and not specific chemical compositions), the chemical composition of a distillate fuel can vary since a product with the desired fuel properties can be formulated in a number of ways. Fuel oils are distinguished from each other based primarily on their boiling point ranges, chemical additives, and uses. However, whether straight run or blended, distillate fuels are produced to meet the ASTM specifications for either Fuel Oils or Diesel Fuel Oils (ASTM 2001, 2002). The ASTM specification for diesel fuels limits the aromatic content of No. 1 D and No. 2-D low sulfur diesel fuels to a maximum 35% by volume (ASTM, 2002).

Figure 1. Gas Oils Process Diagram



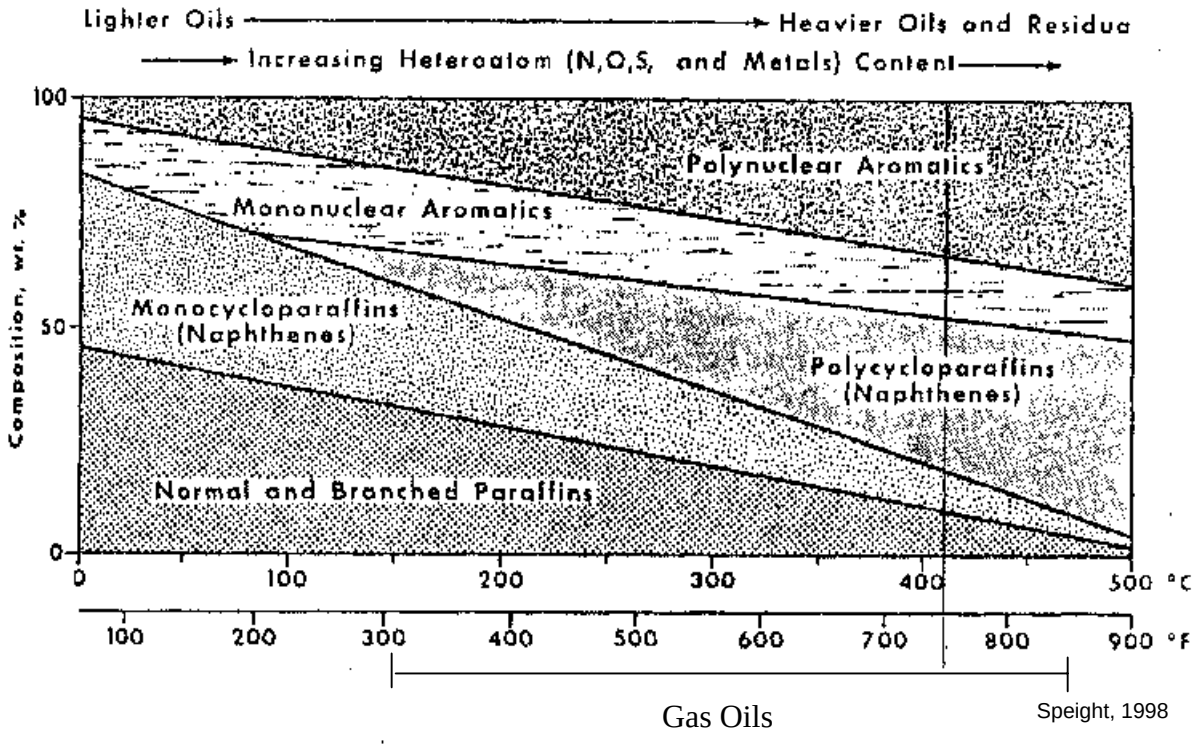
ChevronTexaco, 2003

Note: AGO = atmospheric gas oil
VGO = vacuum gas oil
HCO = heavy cycle oil

The materials in this category are complex petroleum mixtures that boil between 300 and 880ºF (˜150 and 471ºC) and are composed primarily of saturated and/or aromatic hydrocarbons with carbon numbers ranging from C9 to C30. Gas oils contain straight and branched chain alkanes (paraffins), cycloalkanes (naphthenes), aromatic hydrocarbons and mixed aromatic cycloalkanes. As the boiling ranges of the fractions increase, the levels of polycyclic aromatic compounds (PACs), polycycloparaffins and heteroatoms (N, O, S, and metals) increase, while the levels of paraffins decrease (see Figure 2). Most commercial gas oils contain polycyclic aromatic compounds (PACs). In straight-run gas oil components these are mainly 2 and 3-ring compounds, with relatively low concentrations of 4 to 6-ring PACs. The use of heavier atmospheric, vacuum or cracked gas oil components is likely to result in an increase in the content of 4 to 6-ring PACs, some of which are known to be carcinogenic (CONCAWE, 1996). Blended distillate fuels, in addition to containing the hydrocarbons from their blending stocks, may also contain low concentrations of performance additives such as flow improvers, corrosion inhibitors, defoamers, dyes/markers, anti-oxidants, stability improvers, cetane improvers, detergents and anti-static additives.

Links to additional resources on refining processes and petroleum-related glossaries are presented in Appendix B.

Figure 2. Refinery Stream Composition – Boiling Range vs. General Composition



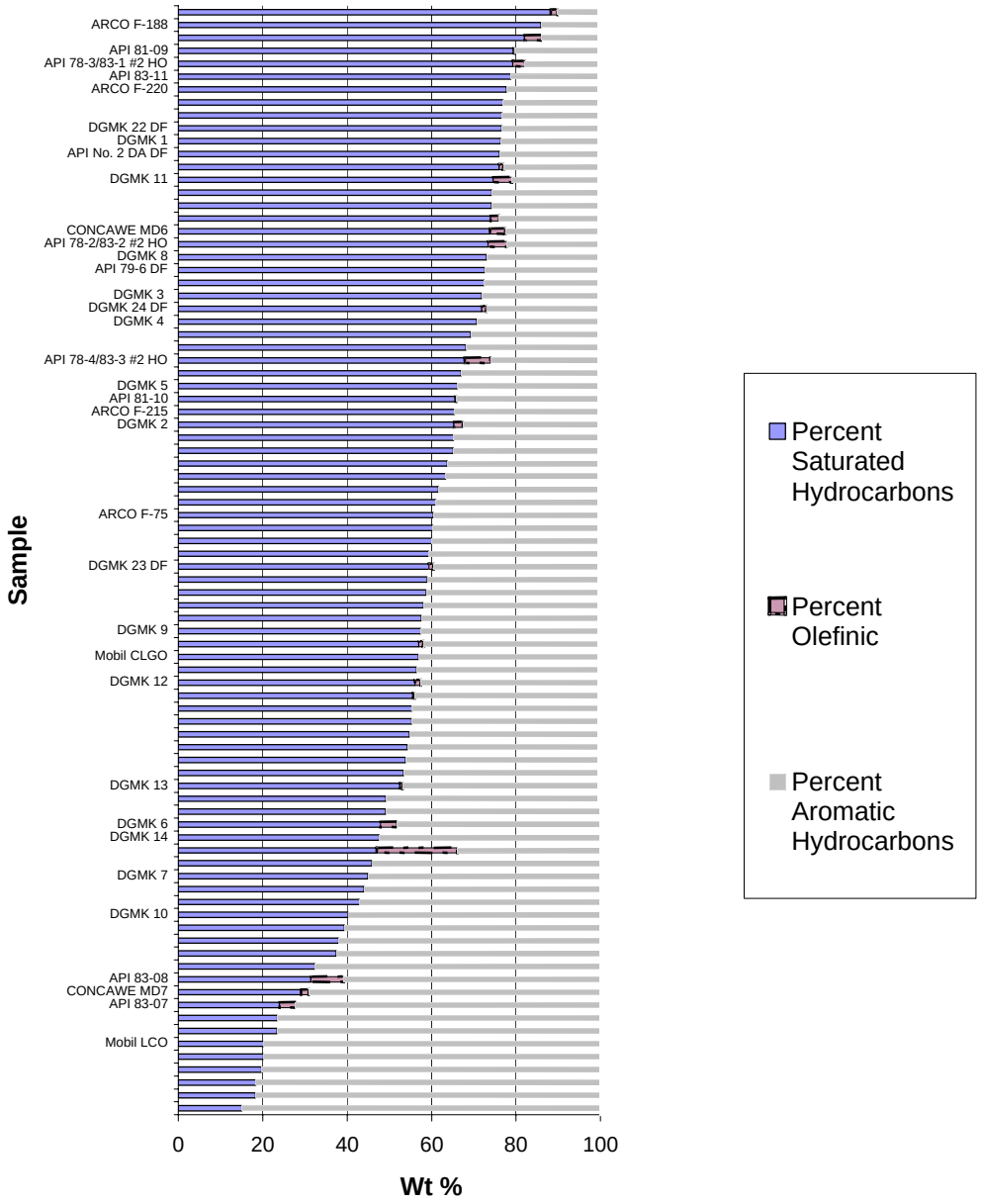
Because they are complex mixtures, the materials in this category are typically not defined by detailed compositional information but instead by process history, physical properties, and product use specifications (ASTM 2001, 2002). Whereas detailed compositional information may be limited, general compositional information can be inferred from the gas oil's physical properties and the type of processing it has undergone, e.g. the higher the boiling temperature range of a fraction, the higher the molecular weight of the oil's components. Similarly, streams that have been "cracked" have higher olefin and aromatic hydrocarbon content while straight run gas oil streams that have undergone a limited amount of additional processing are composed predominantly of saturated hydrocarbons.

Compositional information on 86 gas oil samples (representing 15 of the CAS numbers in this category) showed that the range of hydrocarbon types was:

Olefins: 0 – 19.0%
Saturates: 18 - 86%
Aromatics: 14 - 82 %

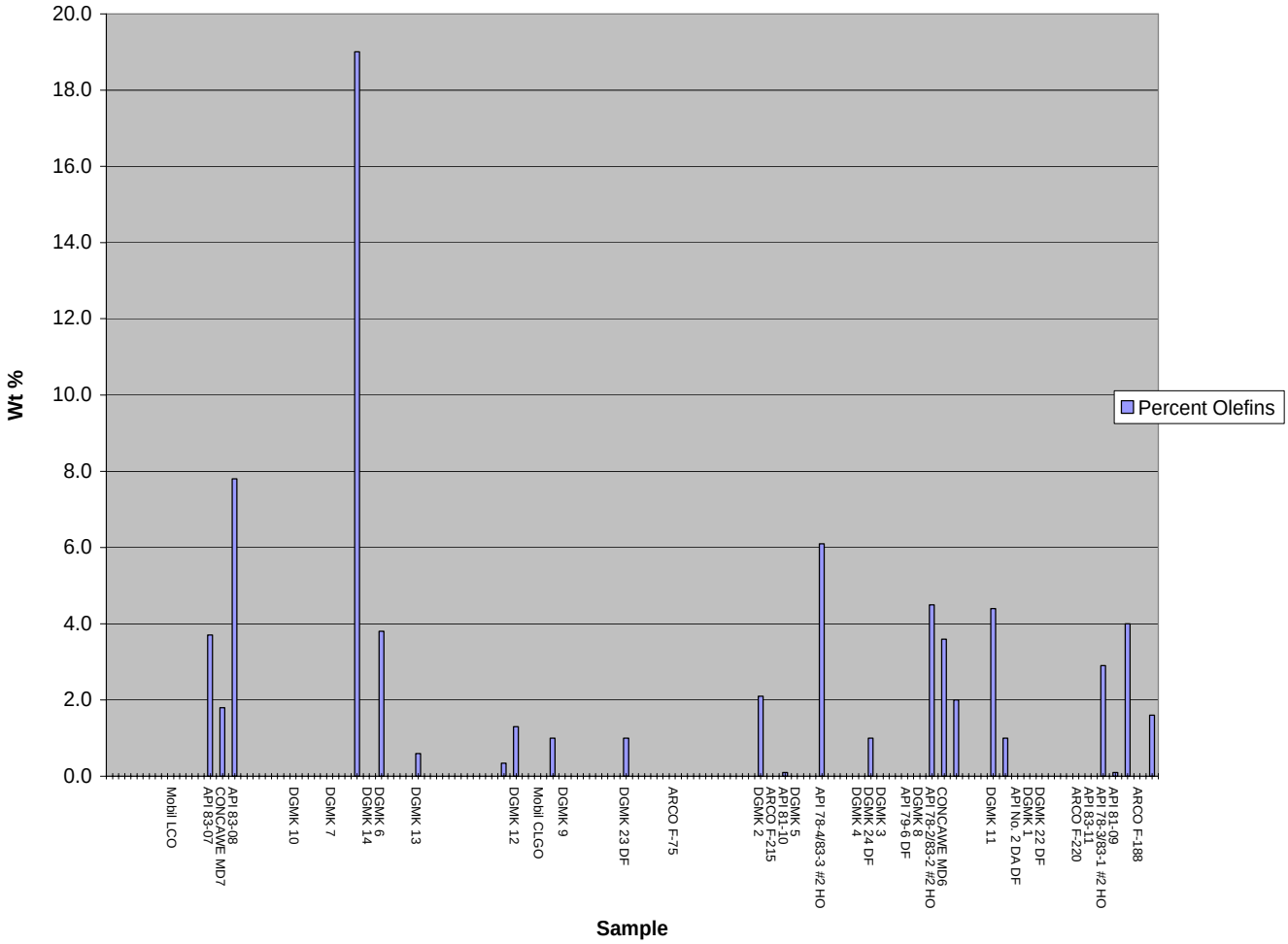
As shown in Figure 3, the saturate and aromatic hydrocarbon content of the Gas Oil category members forms a continuum from high saturate content to high aromatic content. Of the 86 samples graphed in Figure 3, one was reported to have an olefin content of 19%. As shown in Figure 4, this was a higher olefin content than any of the other 81 samples, which were all less than 8%, the majority containing less than 2% olefins. Olefins occur at very low levels (if at all) in crude oil, but may be found in higher concentrations in refinery streams that have undergone cracking reactions. The Testing Group thinks the 19%, while not unrealistic, is not representative of the olefin levels found in the majority of the materials within this category. The American Chemistry Councils Higher Olefins Panel's Test Plan on Higher Olefins and the API HPV Consortium's on Gasoline Blending Streams Test Plan both contain information regarding olefin toxicity.

Figure 3. Composition of Representative Samples of Gas Oils and Distillate Fuels



Note: Samples shown with a descriptive title, i.e. “ARCO F-188”, are from studies described in the robust summary (Appendix C). Compositional information for 86 samples of gas oils and distillate fuels was obtained from the published literature and relevant company reports.

Figure 4. Olefin Content of Representative Samples of Gas Oils and Distillate Fuels



Note: Samples shown with a descriptive title, i.e. “ARCO F-188”, are from studies described in the robust summary (Appendix C). Compositional information for 86 samples of gas oils and distillate fuels was obtained from the published literature and relevant company reports.

Category Rationale and Test Material Description

The Testing Group made the following assumptions when analyzing the existing data, proposing testing and identifying potential test materials:

- The materials included in the Gas Oils category are related from both process and physical-chemical perspectives;
- Chemical-oriented groupings based on two primary chemical classes, saturated and aromatic hydrocarbons, can be used to characterize the range of materials found in this category;
- The saturated and aromatic hydrocarbon content of the category members forms a continuum from high saturate content to high aromatic content;
- The properties (physical-chemical properties and toxicities) of the streams in the category are bounded compositionally by streams that are predominantly saturated hydrocarbons and streams that are predominantly aromatic hydrocarbons;

- Knowledge of the biological activity of representative gas oil streams "enriched" in either saturated or aromatic hydrocarbons, combined with data on the distillate fuels, make it possible to predict the toxicity potential for untested gas oil streams with defined saturated or aromatic hydrocarbon characteristics;
- Key parameters when analyzing this category are the percentage of aromatic and saturated hydrocarbons, and for some mammalian endpoints (developmental, reproductive, mutagenic) 3-7 ring polycyclic aromatic compounds (PAC) content.

The Testing Group is proposing to perform mammalian toxicity studies on two gas oil samples that represent the extremes of hydrocarbon composition found in this category; one that has a relatively high saturated hydrocarbons content and one that has a relatively high aromatic hydrocarbons content (see Figure 3 for potential compositional values). Mammalian testing will also be conducted on a representative sample of a No. 2 distillate fuel, the substance in this category with the greatest potential for public exposure.

Because the compositions of the materials in this category vary over time, it is not possible to specify in this Test Plan the exact chemical makeup of the test samples that will be used in the mammalian testing. However, specific analytical data on the test samples will be developed and made available when the samples are obtained. The Testing Group will attempt to maximize each of the test samples 3-7 ring polycyclic aromatic compound (PAC) content. This is being done because of the indications in the existing published literature that the mutagenic, carcinogenic and developmental toxicity of these materials correlate with their 3-7 ring PAC content (Feuston, 1994). The Testing Group also recognizes that while the olefin content of these materials is generally low, selected streams may have measurable levels of olefins. Consequently, the Testing Group will attempt to maximize the olefin content of the high aromatic hydrocarbon sample that is selected for mammalian toxicity testing. This information will supplement the data on olefins contained in the American Chemistry Councils Higher Olefins Panel's Test Plan on Higher Olefins and the API HPV Consortium's on Gasoline Blending Streams Test Plan.

The Testing Group is also proposing to perform biodegradability and aquatic toxicity testing of two gas oil samples. Samples will be chosen that represent the extremes of hydrocarbon composition found in this category; one having a relatively high saturated hydrocarbons content and one having a relatively high aromatic hydrocarbons content. Candidate gas oil streams also will be evaluated for their potential to produce the greatest water soluble fraction of hydrocarbons in aqueous solutions (i.e., low carbon number). In this way the boundaries of the saturated/aromatic hydrocarbons spectrum will be tested using samples expected to produce the greatest bioavailable fraction of petroleum hydrocarbons. Thus, the samples that will be selected for ecotoxicity testing may not be the same samples used for mammalian testing. Existing biodegradation and aquatic toxicity test data on distillate fuels are expected to fall within the range of the biodegradation and toxicity values of the two gas oil streams.

Evaluation of Existing Health Effects Data and Proposed Testing

General Evaluation

Many studies have been reported for this category of materials, ranging from acute to long-term carcinogenicity studies. Additional reviews by various expert panels have also been published (ATSDR, 1995; CONCAWE, 1991, 1996, 2001; IARC, 1988). Because fuel oils and transportation fuels of the same grade (e.g. No. 2 home heating oil and No. 2 diesel fuel) are virtually indistinguishable on the basis of their gross physical or chemical properties (IARC, 1988), data generated on either material can be used to characterize the toxicity of both materials.

The Test Plan addresses the health effects endpoints of the category by:

- Evaluating the extensive toxicology database for the gas oil refinery streams and the distillate fuels,
- Using read-across information whenever possible among category members, and
- Proposing the minimal amount of toxicity testing needed to characterize the category boundaries and test the hypothesis that the reproductive toxicity of a gas oil stream correlates to its 3-7 ring PAC content.

Acute Toxicity

Gas Oil Streams

- **Streams Composed Predominantly of Aromatic Hydrocarbons**
  Oral LD_{50}s for two gas oils (72.4% & 60.8% aromatic hydrocarbons) ranged from 3.2 to 7.18 g/kg_{bw} (API, 1982a; 1985d). The dermal LD_{50}s of the two gas oils were reported to be >2.0 $g//kg_{bw}$ (API, 1982a; 1985d). Inhalation LC_{50} values for the same two samples were 5.4 and 4.65 mg/l (API, 1986a,b). When tested in rabbits for skin irritation, both streams produced "moderate" to "severe" irritation (API, 1982a; 1985d). When tested for eye irritation in rabbits, the two gas oils produced Draize scores of 1.7-3.2 and 0-2.0 (unwashed and washed eyes) at 24 hours (API, 1982a; 1985d). The same two materials were reported to be "not sensitizing" when tested in guinea pigs (API, 1982a, 1985d). A skin irritation test on a third sample (69.1% aromatic hydrocarbons) produced "moderate" to "severe" erythema in two of three animals at 60 minutes (Exxon, 1996b).

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  Oral and dermal LD_{50}s of three samples of gas oils (65.6% - 79.4% saturated hydrocarbons) were >5 g/kg_{bw} and >2.0 g/kg_{bw}, respectively (API, 1982b,c; 1985e). In all the studies, clinical signs were similar irrespective of the test material. Inhalation LC_{50} values for the same three samples ranged from 1.78 to 7.64 mg/l (API, 1983a,b; 1987a). In rabbits, the three materials were reported to produce "moderate" to "severe" irritation on both intact and abraded skin (API, 1982b,c; 1985e). When tested for eye irritation in rabbits, the three gas oils produced Draize scores of 1.0 – 2.0 and 0.0 (unwashed/washed eyes) at 24 hours (API, 1982b,c; 1985e). The three materials, when tested in guinea pigs, were not skin sensitizers (API, 1984b,c; 1985e). An additional sample of a gas oil (73.7% saturated hydrocarbons), when tested for dermal irritation elicited "minimal" transient irritation (Exxon, 1996b).

Distillate Fuels
The oral LD_{50} in Sprague-Dawley rats of a market-place sample of diesel fuel (72.6% saturated hydrocarbons) was found to be 9.0 ml/kg_{bw} (95% confidence interval of 5.58 to 14.51 ml/kg) (API, 1980d). The dermal LD_{50} in New Zealand white rabbits of the same test material was reported to be greater than 5 ml/kg_{bw} (API, 1980d). When tested for skin irritation, the same market place sample was judged "extremely irritating" (API, 1980d). However, the same sample was found to be non-irritating and non-sensitizing in an eye irritation study in rabbits and a sensitization study in guinea pigs, respectively (API, 1980d).

Oral LD_{50}s of three samples of home heating oils (containing 67.8% - 79.2% saturated hydrocarbons) ranged from 14.5 to 21.2 ml/kg_{bw}, while the dermal LD_{50}s were all >5 gm/kg_{bw} (API, 1980a,b,c). In rabbits, the three materials produced primary skin irritation scores ranging from 3.37 to 3.98 (API, 1980a,b,c). When tested for eye irritation in rabbits, the three home heating oils produced Draize scores of 0 – 0.7 (rinsed eyes) and 0 –1.33 (unrinsed eyes) (API, 1980a,b,c).

Summary: No additional testing is planned. Multiple acute toxicity studies have been reported on both high saturate and high aromatic gas oil refinery streams high in either saturated or aromatic hydrocarbons, as well as on blended distillate fuels. The Testing Group thinks the existing data is sufficient to characterize the acute toxicities of this category of materials.

Repeat-Dose Toxicity

Six 28-day and two 90-day dermal studies have been reported for various gas oils. Two 28-day inhalation studies have also been conducted. In preparing this Test Plan, the approach of the Testing Group has been to review the available toxicology studies and include in the robust summary a detailed description(s) of the one study or a small number of studies that best address each SIDS Level 1 endpoint. Other studies are cited in the appropriate "Remarks" section of the Robust Summary and are intended to supplement the readers' knowledge. Therefore, the attached Gas Oils Robust Summary (Appendix C) contains complete summaries for two 28-day and both 90-day studies done via the dermal route. Results from the remaining four 28-day dermal studies are summarized on pages 34-35 of the Gas Oils Robust Summary. Complete summaries have also been included in the Robust Summary for the two 28-day inhalation studies.

Gas Oils Streams

- **Streams Composed Predominantly of Aromatic Hydrocarbons**
  Dose levels of 8, 25,125, 500 or 1,250 mg/kg$_{bw}$/day of a light cycle oil (distillates, light catalytic cracked; 79.8% aromatic hydrocarbons) were applied undiluted to the skin of male and female rats (Mobil, 1985). The test material was applied 5x/week for up to 13 weeks. After two weeks of exposure, the rats dosed with 1,250 mg/kg$_{bw}$/day were terminated due to poor growth and appearance. Rats dosed with 500 mg/kg$_{bw}$/day for 13 weeks had marked reductions in body weight (males only) and thymus size and weight, with decreased lymphocytes in the thymus. Males were more affected than females. Males dosed at 125 mg/kg$_{bw}$/day for 13 weeks had only slightly reduced body and thymus weights. The investigators judged the thymus weight differences to be attributable to a depletion of lymphocytes within the thymus. The livers of both males and females in the 500 mg/kg$_{bw}$/day groups were slightly larger than controls. The 500 mg/kg$_{bw}$/day males were found to have more fat in the liver cells than the controls. At the sites of application, light cycle oil caused marked, persistent effects including severe erythema and edema with visibly thick, stiffened skin. Microscopic examination of the skin from the 500 mg/kg$_{bw}$/day group revealed moderate chronic inflammatory changes of the skin and hair follicles. The investigators judged the NOAELs for males and females to be 25 and 125 mg/kg$_{bw}$/day, respectively.

  In a different study, a light catalytic cracked gas oil (72.4% aromatic hydrocarbons) was tested in a four-week dermal study using rabbits (API, 1985a). Undiluted test material was applied 3x/week to the skin of 5 male and 5 female rabbits at dose levels of 250, 500 and 1000 mg/kg$_{bw}$. Aside from skin irritation, there were no treatment-related clinical signs during the study. Mean body weights were also not affected by the test material. Treatment related skin irritation (up to “severe”) was seen at the site of application. There were no treatment-related effects on hematological or clinical chemical values, or on absolute or relative organ weights. The only gross pathology findings that were treatment-related were skin changes found at the site of test material application. Microscopic examination of tissues from high dose group animals surviving to termination found moderate to severe proliferative and inflammatory changes in the skin of all animals. Associated with these skin changes was an increased granulopoiesis of the bone marrow, attributed to the stress of the severe skin irritation.

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  A gas oil containing 85.86% saturated hydrocarbons was applied daily, 5x/week for 4 weeks to the skins of Sprague-Dawley rats (UBTL, 1992). Dose levels used included 0.05, 0.25 & 1.0 ml/kg$_{bw}$/day. No mortalities or dose-related clinical observations (except for skin irritation) were seen during the study. Body weights were unaffected by exposure to the test material. A dose-related increase in the degree of skin irritation was reported. The only treatment-related finding at necropsy was skin irritation. No treatment related effects were seen on a variety of hematological and clinical chemistry parameters. Furthermore, organ weights, organ weight/body weight ratios and organ/brain weight ratios were unaffected by exposure to the test material. Histological examination of tissues from the control and high-dose groups found no compound-related effects, other than dermal effects related to the irritating properties of the test material. The report authors concluded that the NOAELs for skin irritation and systemic toxicity were <0.05 ml/kg$_{bw}$/day and 1 ml/kg$_{bw}$/day, respectively.

  A coker light gas oil (56.9% saturated hydrocarbons) was applied to the skin of male and female rats at dose levels of 30, 125, 500 and 2000 mg/kg$_{bw}$ (Mobil, 1991). The test material was applied 5 days each week for 13 weeks to animals in the 30 and 125 mg/kg$_{bw}$ groups. Due to skin irritation and moribund conditions, the animals in the 500 and 2000 mg/kg$_{bw}$ groups were sacrificed during weeks 9 and 2, respectively.

  During the weeks they were on study, perineal staining and skin irritation (generally severe) were seen in all treated animals. The body weights of the 2000 and 500 mg/kg$_{bw}$ groups (both sexes) were significantly less than controls. Body weights of males in the 125 mg/kg$_{bw}$ group were less than controls from day 36 onwards. Clinical chemical analyses at 13 weeks showed apparently compound-related effects on a number of parameters. At 13 weeks, increases in white blood cells and the number of segmented neutrophils were found in both male and female high dose (125 mg/kg$_{bw}$) animals. An increase in lymphocytes was found in both the 125 mg/kg$_{bw}$ group (both sexes) and the 30 mg/kg$_{bw}$ group (females). A reduction of approximately 10% was found in the absolute thymus weights of the

male animals in the 30 mg/kg$_{bw}$ group. In the 125 mg/kg$_{bw}$ group (males and females) a number of significant differences in absolute and relative weights were noted for several organs. The primary treatment-related changes seen during the histopathological examinations were severe skin irritation and slight effects on bone marrow and kidneys. The bone marrow effects included a severe reduction in erythropoietic cells and megakaryocytes (2000 mg/kg$_{bw}$) and changes in megakaryocytes, i.e. larger, vacuolated, and/or darkened nuclei and/or clumped cell effects (2000, 500 and 125 mg/kg$_{bw}$). Kidney effects included basophilia in the tubular cortex (predominantly in males), focal inflammation, and dilation of ducts in the medulla and tubules in the cortex.

Two gas oils (79.4% & 65.6% saturated hydrocarbons) have been tested in a four-week inhalation study (API, 1986f). In the study, Sprague-Dawley rats were exposed to aerosols of each of the gas oils at a nominal concentration of 25 mg/m^3. Exposures were for 6 hours/day, 5 days/week. For both materials, there were no treatment-related body weight changes or clinical observations. Apart from moderately increased leukocyte counts in animals exposed to the material containing 65.6% saturated hydrocarbons, no treatment related effects were found in any of the hematological or clinical chemical parameters that were measured. Nor were there any test material-related macroscopic pathology findings for either material. Subacute inflammation of the respiratory mucosa lining (trace – mild rhinitis) was observed microscopically in some of the animals exposed to the gas oil containing 65.6% saturated hydrocarbons.

Distillate Fuels

Diesel fuel No. 2 (60.41% saturated hydrocarbons) was applied daily, 5x/week for four weeks, to the skin of Sprague-Dawley rats (UBTL, 1986). The material was applied at dose levels of 0.5, 2.0 and 5.0 ml/kg$_{bw}$/day. There were no deaths or any other treatment-related effects observed during the study, with the exception of an effect on body weights and the occurrence of skin irritation. After the second week of the study, the body weights of the mid- and high dose males were less than those of the controls, with the difference persisting throughout the study. At the end of the study the weight gains of the mid and high dose males were 43% and 13% respectively of those of the controls. Skin irritation occurred at all dose levels, ranging from moderate (low dose) to severe (mid and high dose).

A diesel fuel was applied to the skin of New Zealand white rabbits 5 days/week for 3 weeks at dose levels of 0.2, 0.67 and 2.0 g/kg$_{bw}$/day (IITRI, 1984). Severe skin irritation was seen in all the dosed groups. One of ten males and two of the ten females in the highest dose group died prematurely. A variety of compound-related effects were seen.

One, 3 & 10 ml/kg$_{bw}$/day of a No. 2 home heating oil (67.8% saturated hydrocarbons) was applied undiluted to the skin of male and female New Zealand white rabbits (API, 1980c). The test material was applied daily for 5 days, the animals were given a two day dose-free rest and then the test material was applied daily for an additional 5 days. Severe skin irritation was seen at all dose levels. Two of eight and 7/8 animals died prematurely in the 3 and 10 ml/kg$_{bw}$ /day groups, respectively. The only significant histological findings were those associated with the severe skin lesions.

Two additional samples of home heating oils (containing 79.2% and 73.4% saturated hydrocarbons) have been tested for repeat-dose toxicity (API, 1980a,b). In these studies, material was applied to the skin of rabbits for two weeks. Doses in the first study were 2.5, 4 and 10 ml/kg$_{bw}$/day, while those in the second study 1, 2.5 and 10 ml/kg$_{bw}$/day. Both materials produced severe skin irritation at all dose levels. In the first study, 8/8 animals receiving 10 /kg$_{bw}$/day died prematurely. In the second study, 1/8 and 6/8 animals died prematurely in the 2.5 and 10 ml/kg$_{bw}$/day groups, respectively.

The market-place sample of diesel fuel that was summarized in the acute toxicity section was also tested in a two week repeat-dose study (API, 1980d). Applied to the skin of rabbits for two weeks at dose levels of 4 and 8 ml/kg$_{bw}$/day, the material produced a 67% mortality rate in the 8 ml/kg$_{bw}$/day group.

Carcinogenicity

In addition to the repeat-dose studies discussed above, a number of dermal carcinogenicity studies have been performed on gas oils and distillate fuels. Although carcinogenicity is not a required endpoint of the HPV

program, the Testing Group believes the results may be useful in evaluating the repeated dose endpoint. These studies have been fully summarized and reviewed elsewhere (ATSDR, 1995; CONCAWE, 1996; IARC, 1988). The general conclusions that can be drawn from the animal carcinogenicity studies are:

- Gas oils and distillate fuels are potential skin carcinogens after repeated skin application.
- When applied repeatedly to the skin, carcinogenic gas oils and distillate fuels are associated only with skin tumors and not with an increase in systemic tumors.
- The skin carcinogenicity of the gas oils and distillate fuels correlates with 3-7 ring PAC content.
- Skin tumors produced by materials containing low or no PAC is likely due to a non-genotoxic promotion effect and only observed in the presence of sustained severe skin irritation.

Gas Oils Streams

- **Streams Composed Predominantly of Aromatic Hydrocarbons**
  A cracked gas oil (69.7% aromatic hydrocarbons) was applied to the skins of male C3H mice 2, 4 or 7 days/week for 104 weeks (Exxon, 1996a). The test material was applied either undiluted or at 50% or 28.5% dilutions in mineral oil. The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. Thus, the 100% animals were dosed 2x/week, while the 50% and 28.5% groups were dosed 4x/week and 7x/week respectively. Survival was less in the treated groups compared to the negative controls; at the lower two concentrations (28.5 and 50 %) the difference was statistically significant. Dermal irritation occurred in the groups exposed to the gas oil, scores ranging from 0.0 to 4.0. There were no other treatment-related clinical findings. Treatment related findings at post mortem were limited to dermal irritation. A variety of skin tumors developed in the positive control and gas oil treated groups. Tumor types found included squamous cell carcinomas, fibrosarcomas, melanoma (only 1 treated animal) and papillomas.

  Two samples of gas oils with a high aromatic content (48.3% & 55.1% aromatic hydrocarbons) have been tested in an initiation-promotion assay in male CD-1 mice (DGMK, 1993). Animal survivals were not effected by exposure to the gas oil samples. During both the initiation and promotion phases, the gas oil samples caused slight to moderate skin irritation which was found to be reversible. There were no other treatment-related clinical findings. Of the two samples, both appeared to have weak initiating potentials, while only one showed a weak promoting effect.

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  A straight run, hydrotreated gas oil (73.8% saturated hydrocarbons) was applied to the skins of male C3H mice 2, 4 or 7 days/week for 104 weeks (Exxon, 1996a). The test material was applied either undiluted or at 50% or 28.5% dilutions in mineral oil. The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. Thus, the 100% animals were dosed 2x/week, while the 50% and 28.5% groups were dosed 4x/week and 7x/week respectively. Survival figures of the gas oil treated groups were comparable to that seen in the negative control group. Dermal irritation scores in the gas oil groups ranged from 0.0 to 4.0. There were no other treatment-related clinical findings. Dermal irritation was the only treatment-related finding at post mortem. A variety of skin tumors developed in the positive control and the 100% and 28.5% gas oil groups. The tumor incidence was highest in the group in which skin irritation was greatest. The incidence of the tumors in the gas oil groups was much lower than that seen in the study of a cracked gas oil described above.

  Three gas oils with high saturated hydrocarbon contents (57.5% - 76.4%) have been tested in an initiation-promotion assay in male CD-1 mice (DGMK, 1993). Animal survivals were not effected by exposure to the gas oil samples. During both the initiation and promotion phases, two of the three gas oil samples caused reversible, slight to moderate skin irritation. There were no other treatment-related clinical findings. Of the three samples, two appeared to have weak, if any initiating potentials. And of those two materials, one had a very weak, if any promoting potential.

Distillate Fuels

A dermal carcinogenicity study of a diesel fuel (saturate content unknown) in C3H mice has been reported by IITRI (IITRI, 1985). Over the lifetime of the animals, 50µl of undiluted test material was applied 2x/week to the

shaved skin of male mice. There was a significant increase in the incidence of malignant skin tumors (squamous cell carcinoma or fibrosarcoma) in the treated mice compared to the controls. Other lesions of the treated skin included sloughing of the skin and lesions resembling infection, both of which were seen more frequently in the treated animals.

A sample of a diesel fuel (76.6% saturated hydrocarbons) has been tested in an initiation-promotion assay in male CD-1 mice (DGMK, 1993). Animal survivals were not effected by exposure to the diesel fuel. The study's authors concluded that the diesel fuel sample might be a promoter.

Summary: No additional repeat-dose testing is planned for either gas oils or distillate fuels. Multiple repeat-dose toxicity studies have been reported on a variety of gas oils and distillate fuels. Petroleum hydrocarbons, and specifically gas oils and distillate fuels have been shown to produce dermal and systemic effects when applied dermally. The Testing Group thinks the existing data are sufficient to characterize the repeat-dose toxicity of this category of materials.

***In-Vitro* (Mutagenicity)**

Gas Oils Streams

- **Streams Composed Predominantly of Aromatics Hydrocarbons**
  Modified Ames assays on three gas oils (52.4% - 59.8% aromatic hydrocarbons) produced mutagenicity indices ranging from 7.6 to 9.3 (DGMK, 1991).

  A sample of a light catalytic cracked gas distillate (72.4% aromatic hydrocarbons) produced mixed results in a mouse lymphoma assay (API, 1985i). The report's authors concluded that the sample produced a positive response in the presence of S-9 activation but was not mutagenic in the absence of activation. A second mouse lymphoma assay on a different sample of light catalytic cracked distillate (60.8% aromatic hydrocarbons) produced a positive response both with and without S9 activation (API, 1985f). Testing of a separate aromatic fraction from another gas oil sample produced negative results with and without S9 activation (API, 1987b).

  A sample of a light catalytic cracked gas distillate (72.4% aromatics) was tested with and without S9 activation in the sister chromatid exchange (SCE) assay using Chinese Hamster Ovary (CHO) cells (API, 1988b). While the investigators did find an increase in SCEs above the spontaneous background level, there was no clear dose-response. Consequently the report concluded that the light catalytic cracked gas distillate test sample was equivocal in the test system.

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  Results of modified Ames assays on eleven gas oils (saturates content 51.7% to 79.0%) were reported by DGMK (1991). The eleven test samples had mutagenicity indices ranging from 0.7 to 4.0.

  Three samples of gas oil streams (65.6 - 79.4% saturated hydrocarbons) have been tested in the mouse lymphoma assay, with and without activation (API, 1984a;1985g,h,j;1986d;1987e). Two of the samples have undergone multiple tests. The six separate assays on these three samples have produced, with one exception, positive results, with or without S9 activation. Testing of a separate saturate fraction from one of the samples produced negative results with and without S9 activation (API, 1987c).

  A sample of a hydrodesulfurized middle distillate (65.6% saturates) was tested with and without S9 activation in the sister chromatid exchange (SCE) assay using Chinese Hamster Ovary (CHO) cells (API, 1988c). While the sample was negative without S9 activation, when tested with S9 activation, the results were equivocal, with no clear dose-response.

Distillate Fuels
Results of modified Ames assays on three diesel fuels (59.4% - 76.6% saturated hydrocarbons) were reported by DGMK (1991). The three test samples had mutagenicity indices ranging from 1.7 to 3.9.

An additional Ames assay has been reported on a diesel fuel sample (API, 1978). The diesel fuel was negative in these assays, with and without rat liver S9 activation. The same report also included the results of a mouse lymphoma assay on a No. 2 –D fuel (76.1% saturated hydrocarbons) (API, 1978). With and without activation the test material was negative. However, a sample of home heating oil (67.8% saturated hydrocarbons) was positive with and without S9 activation (API, 1979a).

***In-Vivo* (Chromosomal Aberrations)**

Gas Oils Streams

- **Streams Composed Predominantly of Aromatic Hydrocarbons**
  An *in vivo* bone marrow cytogenetic test in Sprague-Dawley rats has been conducted on light catalytic cracked distillate (72.4% aromatic hydrocarbons) (API, 1986e). The study was conducted according to the OECD Guideline No. 475. The authors of the study report concluded the test material did not induce a significant increase in the percentage of aberrant cells in either male or female animals. This same test material was positive when tested in an *in vivo* sister chromatid exchange assay using mice (API, 1989). A second *in vivo* bone marrow cytogenetic test on a different sample of light catalytic cracked distillate (60.8% aromatic hydrocarbons) was also negative (API, 1985b).

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  Three samples of gas oil streams (65.6% - 79.4% saturated hydrocarbons) have been tested in *in vivo* bone marrow cytogenetic tests using Sprague-Dawley rats (API, 1984a; 1985c,h; 1986c). The assays on these three samples produced consistently negative results. One of the samples (65.6% saturated hydrocarbons) also produced negative results when tested in an *in vivo* sister chromatid exchange assay using mice (API, 1988a).

Distillate Fuels
A sample of a No. 2-distillate fuel (76.1% saturated hydrocarbons) has been tested in an *in vivo* bone marrow cytogenetic test using male rats (API, 1978). Similar aberrations were observed in both the treatment and negative control groups, the only difference being the frequency of the aberrations. Based on the total number of cells with aberrations and the mean percent aberrations, the report concluded that at dose levels of 2 and 6 ml/kg_{bw} diesel fuel was clastogenic.

The same distillate fuel was also tested in a dominant lethal assay in male CD-1 mice (API, 1980e). Male mice were exposed by inhalation to diesel fuel at airborne concentrations of 100 and 400 ppm. Exposures were for 6 hours a day, 5 days per week for 8 weeks (40 doses). Following completion of the exposures, the males were sequentially mated to two females per week for 2 weeks. Two weeks after the middle of their mating week, females were killed and necropsied and the uteri examined. A variety of reproductive parameters were calculated. The report concluded that the test material did not cause any significant pre- or post implantation losses when compared to the negative control.

Summary: No additional testing is planned. Existing *in vitro, in vivo* and carcinogenicity studies are adequate to characterize the genotoxicity of gas oils and distillate fuels.

Reproductive/Developmental Toxicity

Gas Oils Streams

- **Streams Composed Predominantly of Aromatic Hydrocarbons**
  In a developmental toxicity study, a light cycle oil (79.8% aromatic hydrocarbons) was applied daily to the skin of pregnant Sprague-Dawley rats on days 0-19 of gestation (Mobil, 1988). Dose levels administered included: 25, 50, 125, 250, 500 and 1000 mg/kg_{bw}/day. All animals were euthanized on day 20. In the dams, erythema and flaking of the skin were observed in all gas oil exposed groups. Skin effects were observed in all but the 25 mg/kg_{bw} group. At doses greater than 25 mg/kg_{bw} there was a decrease in body weight and body weight gain compared to the controls, with an accompanying reduction in food consumption. There were no treatment-related findings at necropsy. Blood levels of triglycerides were increased in a dose-related manner in the 250, 500 and 1000 mg/kg_{bw} groups. Fetal

body weights were reduced in the 500 and 1000 mg/kg$_{bw}$ groups, with only the reduction in the 1000 mg/kg$_{bw}$ group being statistical significant. There were no significant increases in resorptions, soft tissue variations and malformations, and skeletal malformations in any of the dose groups.

- **Streams Composed Predominantly of Saturated Hydrocarbons**
  A developmental toxicity screening study has been reported on a gas oil composed of 65.4% saturated hydrocarbons (UBTL, 1994a). Undiluted test material was applied daily on days 0 to 20 of gestation to the clipped skin of resumed-pregnant female rats. Dose levels included 50, 150 and 500 mg/kg$_{bw}$/day. Signs of maternal toxicity considered by the study director to be related to administration of the test material included decreased body weights and body weight changes at a dose of 500.0 mg/kg$_{bw}$. Test material related dermal irritation was observed in all of the dose groups. Signs of developmental toxicity considered to be related to administration of the test material included lower pup body weights on Lactation Days 0 and 4 at doses of 150.0 and 500.0 mg/kg. The proportion of pups surviving to Lactation Day 4 was decreased at a dose of 500.0 mg/kg. Based on the results of this study, the study director concluded the no-observable-effect level (NOAEL) for maternal toxicity was less than 50.0 mg/kg$_{bw}$ (dermal irritation at 50.0 mg/kg$_{bw}$ and decreased body weight at 500.0 mg/kg$_{bw}$). The no-observable-effect level (NOAEL) for signs of developmental toxicity was 50.0 mg/kg$_{bw}$.

  A second developmental screening study, similar in design to the one described in the preceding paragraph, has been reported on a gas oil containing 77.7% saturated hydrocarbons (UBTL, 1994b). Dose levels in this second study were 125, 250 and 1000 mg/kg$_{bw}$/day. Dams in the 125 and 250 mg/kg$_{bw}$ dose groups were administered test material on days 0-20 of gestation. Dams in the 1000 mg/kg$_{bw}$ were dosed only on days 5-9 of gestation. Signs of maternal toxicity considered by the study authors to be related to be compound-related included dermal irritation (all dose groups), decreased body weights, body weight changes, and food consumption in the 1,000.0 mg/kg$_{bw}$ dose group. The study authors also concluded there were no signs of developmental toxicity related to administration of the test material in any of the dose groups. Based on the results of this study, the no-observable-effect level (NOEL) for maternal toxicity was less than 125 mg/kg (dermal irritation at 125, 250 and 1000 mg/kg; decreased body weights, body weight changes and food consumption at 1000 mg/kg). In the groups of animals administered 125 or 250 mg/kg$_{bw}$ of the test material, the no-observable-effect level (NOEL) for maternal toxicity and signs of developmental toxicity was concluded to be 250.0 mg/kg$_{bw}$. Administration of the test article at a higher dose level (1,000 mg/kg$_{bw}$), but for a shorter dosing period, (Gestation Days 5 to 9), produced signs of maternal toxicity without signs of developmental toxicity.

Distillate Fuels
A developmental toxicity study has been reported on a diesel fuel consisting of 76.1% saturated hydrocarbons (API, 1979b). In the study, groups of presumed-pregnant Sprague-Dawley rats were exposed to nominal atmospheric concentrations of 100 and 400 ppm. Exposures were for 6 hours each day from day 6 through day 15 of gestation. On day 20 all the animals were killed and both dams and fetuses thoroughly examined. One third of the fetuses were fixed for soft tissue examination. The remaining fetuses were examined for skeletal abnormalities. There were no deaths during the study and all animals were normal in appearance throughout. The 400 ppm group had a reduced food intake during days 7-15 of gestation. No treatment-related differences were found in a variety of parameters, including sex ratios of the fetuses, number of implantation sites, resorptions, and live fetuses. With the exception of subcutaneous hematomas that occurred at a higher rate in the test article exposure groups, there were no test article-related abnormalities found in either the soft tissues or skeletons of the fetuses.

Summary: Reproductive/developmental screening studies (OECD 421) will be performed on two representative gas oil samples; one that has a relatively high saturated hydrocarbons content and one that has a relatively high aromatic hydrocarbons content. A No. 2 distillate fuel (the substance in this category with the greatest potential for public exposure) will also be tested in a reproductive/developmental screening study (OECD 421). After reviewing the available data, the Testing Group thinks there is adequate developmental toxicity data on gas oils and distillate fuels. However, none of the developmental studies provides data on pre-mating exposure of males or females. This lack of reproductive toxicity data on gas oils suggests the need for additional studies on representative samples of gas oils that span the range of saturated and aromatic hydrocarbons composition. Similarly, the lack of reproductive data on a distillate fuel and the positive result in the *in vivo* bone marrow cytogenetic assay support the reproductive

toxicity testing of a distillate fuel. The Testing Group thinks the dermal route of exposure is appropriate for use in these studies since:

- the dermal route is a major route of human exposure,
- systemic effects have been seen in numerous studies of petroleum hydrocarbons done using the dermal route of exposure,
- historic data in the rat shows absorption of PACs via both the dermal and oral routes of exposure, and
- the dermal route of exposure minimizes the potential "first pass" metabolism by the liver of the biologically available/active impurities.

However, the Testing Group recognizes that the irritating properties of these materials may adversely affect the conduct of the studies. Therefore, when designing the studies, the Testing Group will include techniques to reduce the dermal irritation and associated stress (e.g., rotating dose sites or mixing the test material with mineral oil) and will consider the results of a pilot study before finalizing the protocols for the full studies.

While the Testing Group is proposing to perform a Reproductive/developmental screening study (OECD 421) on a distillate fuel, the Group is aware that scientists with oil companies in the European Union have also identified the need for a reproductive toxicity study on a distillate fuel. The Testing Group will discuss a joint testing effort with the European group. This may result in a more in depth study protocol than the OECD 421 test being proposed in this Test Plan. The Agency will be notified if a collaborative effort is agreed upon.

Evaluation of Existing Physico-Chemical and Environmental Fate Data and Proposed Testing

The physicochemical endpoints for the EPA HPV chemical program include melting point, boiling point, vapor pressure, octanol/water partition coefficient (log Kow), and water solubility. Environmental fate endpoints include photodegradation, hydrolysis, environmental transport and distribution (fugacity), and biodegradation. Although some data for products in this category exist, not all of these endpoints are defined and a consensus database for chemicals that represent products in this category does not exist. Therefore, calculated and measured representative data have been identified and a technical discussion provided, where appropriate. The EPIWIN© computer model, as discussed in the US EPA document entitled "The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program" has been used to calculate physical-chemical properties of representative constituents of gas oils (EPA, 2000).

The substances covered under this HPV testing plan are mixtures of differing compositions. Because of the diversity of compounds encompassing gas oils, it is not feasible to model the physicochemical endpoints for each potential compound. Rather, modeling efforts were directed towards those hydrocarbon components of the gas oils that would most likely disperse to various environmental media. Since molecular weight and structural conformation determine in large part the solubility and vapor pressure characteristics of the hydrocarbons, representative isomeric structures of the lower (C_9) and higher molecular weight (C_{30}) hydrocarbons of each group of the chemical species found in these materials (paraffinic, naphthenic, olefinic and aromatic) were modeled for relevant physicochemical and fate processes. This provided a range of values that were considered to encompass the majority of the compounds in gas oils.

Physico-Chemical Data

Melting Point

For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe the physical phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM, 1999). The pour point temperature falls as an oil's viscosity increases. Pour point values for a variety of gas oil types have been reported in the literature. The pour point of two samples of a light catalytic-cracked gas oil (60.8% - 79.8% aromatic hydrocarbons) were measured by API (1987d) to be –15 ° and –12 °C. The pour points of various distillate fuels (59.4% - 79.2% saturated hydrocarbons) were found by CONCAWE (1996) to range from –6 ° to 0 °C for an automotive gas oil (diesel), a heating oil, and a marine distillate fuel. The pour point values for four diesel fuels reported by Jokuty et al. (2002) ranged from –50° to –14 °C. The wide range in pour point values

for distillate fuels is likely due to fuel additives that increase the flow characteristics of products distributed to cold regions.

Summary: No additional testing is proposed. The pour points of various gas oils and distillate fuels have been adequately measured.

Boiling Point

Gas oils do not have a single numerical value for boiling point, but rather a boiling or distillation range that reflects the individual components in the hydrocarbon mixture. Distillation ranges for a variety of gas oils have been reported for a number of blended gas oil products (CONCAWE, 1996; Jokuty et al., 2002) and individual gas oil production streams (API, 1987d). Typical distillation ranges for blended fuels are 160 to 390 °C for an automotive gas oil (diesel fuel), 160 to 400 °C for a heating oil, and 170 to 420 °C for a distillate marine fuel (CONCAWE, 1996). Jokuty et al. (2002) gave distillation ranges from three sources of diesel fuel as 141 to 320 °C (diesel fuel – Alaska), 246 to 388 °C (diesel fuel – Canada), and 174 to 352 °C (diesel fuel – southern U.S.A.). Typical low end and high end distillation temperatures for gas oil production streams analyzed by API (1987d) were 172 and 344 °C for a hydrodesulfurized middle distillate (65.6% - 79.4% saturated hydrocarbons), 185 and 391 °C for a straight-run middle distillate (78.8 saturated hydrocarbons), and 185 and 372 °C for a light catalytic cracked distillate (60.8% - 79.8% aromatic hydrocarbons). These values are consistent with the distillation range of 150 to 450 °C described by CONCAWE (1996) for the general category of middle distillate oils having hydrocarbon components predominantly in the range of C_9 to C_{30}. No substantial differences in boiling range were apparent for gas oils with high concentrations of either aromatic or saturated hydrocarbons.

Summary: No additional testing is proposed. The distillation ranges of a variety of gas oils and distillate fuels have been adequately measured.

Vapor Pressure

For mixtures such as petroleum products, the vapor pressure of the mixture is the sum of the partial pressures of the individual components (Dalton's Law of Partial Pressures). Gas oils are expected to have low vapor pressure due to their boiling range (150 to 450 °C) and molecular weights of the constituent hydrocarbons (C_9 – C_{30} carbon atoms). Measured values according to ASTM Method D2889 for automotive gas oil (diesel fuel) and heating oil were approximately 0.4 kPa at 40 °C (CONCAWE, 1996), while measurements made on No. 2 fuel oil and diesel oil according to the Reid Method (ASTM, D323) were reported as 2 kPa at 38 °C (Jokuty et al., 2002). Because the physical-chemical characteristics of distillate fuels reflect the gas oil streams from which they were produced, these vapor pressure measurements are expected to approximate the vapor pressures of individual gas oils. Vapour pressure estimates of constituent hydrocarbons in gas oil streams made using EPIWIN (EPA, 2000) approximated the measured data. Vapour pressure estimates of low molecular weight hydrocarbons of varying isomeric structures fell within a range of 0.01 to 1.6 kPa, with higher molecular weight hydrocarbons showing very low vapour pressures (e.g., 10^{-8} to 10^{-10} kPa).

Summary: No additional testing is proposed. The vapor pressures of representative distillate fuels have been adequately measured. These measurements are expected to approximate the vapor pressures of individual gas oils.

Partition Coefficient

The percent distribution of the hydrocarbon groups (i.e., paraffins, olefins, naphthenes, and aromatics) and the carbon chain lengths of hydrocarbon constituents in gas oils largely determines the partitioning characteristics of the mixture. Generally, hydrocarbon chains with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). Because gas oils are complex mixtures, it is not possible to determine their log K_{ow} values. Rather, partition coefficients have been calculated for individual component hydrocarbons from known hydrocarbon composition (CONCAWE, 1996). Those calculated K_{ow} values ranged from 3.9 to >6.0 for a hydrodesulfurized middle distillate ((65.6% - 79.4% saturated hydrocarbons), straight-run middle distillate (78.8% saturated hydrocarbons), and a light cat-cracked distillate (60.8% - 79.8% aromatic hydrocarbons). Those estimates are in agreement with a range of log K_{ow} values of 3.7 to >6 determined by the Testing Group using EPIWIN (EPA, 2000) for various C_9 to C_{30} hydrocarbon components in gas oils. There are no apparent differences in the range of K_{ow} values determined for gas oils with high concentrations of either aromatic or saturated hydrocarbons.

Summary: No additional modeling is proposed. Partition coefficients (K_{ow}) of 3.9 to >6.0 have been estimated for representative C_9 to C_{30} hydrocarbon components of gas oils. A similar range of partition coefficients would be expected for component hydrocarbons in distillate fuels.

Water Solubility
When released to water, gas oils will float and spread at a rate that is viscosity-dependent. Component hydrocarbons in gas oils will partition to water according to their individual solubility values. For individual hydrocarbon constituents in gas oils, water solubility values vary by orders of magnitude. Molecular weight and chemical structure have a great influence on the ultimate degree of solubility. Water solubility of component hydrocarbon molecules was estimated using EPIWIN©, WSKOW V1.40 computer model (EPA, 2000). Water solubility ranged from essentially insoluble (approximately 10^{-8} mg/L) for the higher molecular weight fractions (e.g., C_{30}) within gas oil to approximately 52 mg/L for a C_9 alkylbenzene.

Summary: **No additional modeling is proposed.** Water solubility values have been calculated for various C_9 to C_{30} PONA constituents in gas oils. A similar range of water solubility values would be expected for component hydrocarbons in distillate fuels.

Environmental Fate Data
The typical battery of tests used to measure the environmental fate of a material is not easily performed on the materials of this category because of their physical and chemical properties. Therefore, when measured data are not available to describe a fate process, components of the gas oils will be modeled where necessary using EPIWIN® (U.S. EPA, 2000).

Photodegradation
The direct aqueous photolysis of an organic molecule occurs when it absorbs sufficient light energy to result in a structural transformation. Only light energy at wavelengths between 290 and 750 nm can result in photochemical transformations in the environment, although absorption is not always sufficient for a chemical to undergo photochemical degradation. Saturated and one-ring aromatic hydrocarbons do not show absorbance in the 290 to 800 nm range and would not be expected to be directly photodegraded. Polyaromatic hydrocarbons, on the other hand, have shown absorbance of the 290 to 800 nm range of light energy and could potentially undergo photolysis reactions. The degree and rate at which these compounds photodegrade depends upon whether conditions allow penetration of light with sufficient energy to effect a change.

Components in gas oils that do not directly photodegrade (e.g., paraffins, naphthenes, and one-ring aromatic compounds) may be subject to indirect photodegradation. Indirect photodegradation is the reaction with photosensitized oxygen in the atmosphere in the form of hydroxyl radicals (OH^-). The potential to undergo indirect photodegradation can be estimated using the atmospheric oxidation potential (AOP) model subroutine (AOPWIN V1.90) in EPIWIN© (EPA, 2000), which calculates a chemical half-life and an overall OH^- reaction rate constant based on a 12-hour day and a given OH^- concentration. Atmospheric oxidation rates and half-lives were calculated for the low and high end of the range of molecular weight constituents of gas oils (e.g., C_9 and C_{30} hydrocarbon structures). AOP half-life estimates for these compounds ranged from 0.1 (for various C9 to C30 olefinic structures and C30 2+ring aromatic compounds) to 1.5 days (for a C9 one-ring aromatic structure). Based on the half-life values calculated by AOPWIN, no substantial differences in indirect photodegradation potential is expected between gas oils with high concentrations of either aromatic or saturated hydrocarbons.

Summary: No additional modeling is proposed. Atmospheric half-lives of 0.1 to 1.5 days have been calculated for representative C_9 and C_{30} hydrocarbon components of gas oils.

Stability in Water
Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). Because gas oils do not contain significant levels of these functional groups, materials in the gas oils category are not subject to hydrolysis.

Summary: No additional modeling is proposed. The materials in the gas oils category do not contain chemical moieties that undergo hydrolysis.

Transport and Distribution in the Environment (Fugacity)
Fugacity-based multimedia modeling can provide basic information on the relative distribution of chemicals between selected environmental compartments (i.e., air, soil, sediment, suspended solids, water and biota). The U.S. EPA has agreed that computer modeling techniques are an appropriate approach to estimating chemical partitioning. A widely used fugacity model is the EQC (Equilibrium Criterion) model. The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized unit environment. EPA cites the use of the EQC model in its document, "Determining the Adequacy of Existing Data", which was prepared as guidance for the HPV chemicals program. The EQC model was used by the Testing Group to estimate the percent distribution in environmental media (i.e., air, water, soil, sediment, and fish) of various C_9 to C_{30} compounds representing the different classes of hydrocarbons found in gas oils (e.g., paraffins, olefins, naphthenes, and aromatics). Hydrocarbons having nine carbon atoms showed a tendency to partition to air (up to 98%). As molecular weight increases, partitioning shifts to soil, which accounts for 98% of the distribution of the C_{30} components. This trend was similar for saturate and aromatic structures alike. Therefore, gas oils with high concentrations of either aromatic or saturated hydrocarbons are expected to partition in the environment in a similar manner.

Summary: No further modeling is proposed. Fugacity modeling has been done to provide an estimate of the percent distribution in environmental media of various C_9 to C_{30} PONA hydrocarbons found in gas oils.

Biodegradation
Little data are available on the behavior of specific gas oils in standard tests for biodegradability. Much of what is known is based on information gained from testing hydrocarbon mixtures of other petroleum products. Under standard biodegradability tests, hydrocarbon compounds representative of those found in gas oils typically do not pass ready biodegradability test conditions. Although those compounds are not recognized as being readily biodegradable, most hydrocarbon species present in gas oils are known to be ultimately degraded by aerobic microorganisms (Connell and Miller, 1980; CONCAWE, 1996). Lower molecular weight compounds may be expected to be degraded relatively quickly in aerobic conditions, while higher molecular weight compounds, particularly polycyclic aromatics, will degrade slower. Much of this evidence is based on bioremediation studies of contaminated soils, which have shown that hydrocarbon components in gas oils are degraded in the presence of oxygen (Hoeppel et al., 1991; Miethe et al., 1994). Bioremediation of a diesel fuel spill has also been demonstrated under Arctic conditions (Liddell et al., 1994).

Evidence of ultimate biodegradability is provided in a study performed on a high aliphatic content gas oil (Exxon, 1994). In that test, gas oil was incubated under aerobic conditions in the presence of an activated sludge inoculum. After 14 days, approximately 42% was degraded, with little or no biodegradation occurring thereafter. Battersby et al. (1992) observed approximately 40% biodegradation for a gas oil in a 28-day modified Sturm procedure. In two biodegradation studies of diesel fuel using the OECD 301F method, Mobil (1999) measured 57.5% biodegradation and Clark et al. (2003) measured 60% biodegradation. Although the level of degradation measured by Clark et al. (2003) achieved the ready biodegradation pass level for mixtures of similar substances (OECD, 2003), most studies have not shown this level of biodegradation.

Under anaerobic conditions, such as anoxic sediments, rates of biodegradation of gas oils components are negligible and the gas oils may persist under those conditions for some time (CONCAWE, 1996; Brown 1989). Degradation then will be dependent on bioturbation or resuspension to provide microbes with access to oxygen.

Summary: Biodegradability assays will be performed on samples of two gas oil streams.
One sample will be from a stream containing a predominance of saturated hydrocarbons while the second sample will be from a stream containing a predominance of aromatic hydrocarbons. The limited test data available on the biodegradability of gas oils underscores the need for these assays, and the results are expected to provide a range of biodegradation rates expected for substances in this category. The Testing Group does not think testing a gas oil containing olefinic hydrocarbons would be worthwhile due to the relatively low levels of olefins in most of the gas oil samples included in this test plan.

Evaluation of Existing Ecotoxicology Data and Proposed Testing

Multiple ecotoxicological studies on heating and transportation fuels (e.g., no. 2 fuel oil and diesel fuel) have been conducted. In general, these commercial distillate fuels show moderate toxicity to aquatic life. LL50 values for fish ranged from 3.2 to 65 mg/L (Exxon, 1998a-c; 1999; Shell, 1995a,b), while EL50 values for invertebrates ranged from 2.0 to 210 mg/L (Exxon, 2001; Fraunhofer, 2000; Shell, 1994, 1995c,d). All studies used exposures to water accommodated fractions of the gas oils. No differences in the sensitivity of fish and invertebrates to no. 2 fuel oil or diesel fuel were noted. In contrast, algal EL50 values were consistently lower for no. 2 fuel oil, suggesting a greater sensitivity of algae to no. 2 fuel oil than to diesel fuel. EL50 values for inhibition of algal growth rate and biomass ranged from 1.8 to 2.9 mg/L for no. 2 fuel oil and from 10 to 78 mg/L for diesel fuel (Exxon, 1998d,e; Shell, 1995 e,f).

Based on compositional data for gas oil refining streams, these substances span a wide compositional matrix including streams with predominantly saturated hydrocarbons and those with predominantly aromatic hydrocarbons. Although the Testing Group considers the aquatic toxicity data for distillate fuels to be adequate for assessing the aquatic hazard of those materials, there are water solubility and partitioning differences between saturated and aromatic hydrocarbons that could affect the aquatic toxicology of gas oil streams high in saturate or aromatic character. Hence, the Testing Group believes a data gap exists for gas oil streams containing either high levels of saturated or aromatic hydrocarbons and proposes to test two gas oils representing the ends of this spectrum. While olefins comprise a separate hydrocarbon group, their percentage of the total hydrocarbon component in gas oils is generally relatively low. This, and the consideration that olefinic hydrocarbons show water solubility and Log P values similar to saturated hydrocarbons of equal carbon numbers, argue that aquatic toxicity will be driven by either the saturated or aromatic fractions. Although gas oil samples targeted for aquatic toxicity testing may contain an olefinic component, it is not expected to play a significant role in defining their aquatic hazard.

Summary: Aquatic toxicity tests will be performed on samples of two gas oil streams. One sample will be from a stream containing a predominance of saturated hydrocarbons while the second sample will be from a stream containing a predominance of aromatic hydrocarbons.

Matrix of Available Data and Proposed Testing

Table 2. Matrix of Available Data and Proposed Testing

	GAS OIL STREAMS		DISTILLATE FUELS
TEST	**PREDOMINANTLY SATURATED HYDROCARBONS**	**PREDOMINANTLY AROMATIC HYDROCARBONS**	
Physical/Chemical Properties			
Melting Point	Adequate	Adequate	Adequate
Boiling Point	Adequate	Adequate	Adequate
Vapor Pressure	Adequate	Adequate	Adequate
Water Solubility	Adequate	Adequate	Adequate
Partition coefficient (log Kow)	Adequate	Adequate	Adequate
Ecotoxicity			
Algae Growth Inhibition	Test	Test	Adequate
Acute Freshwater Invertebrate	Test	Test	Adequate
Acute Freshwater Fish	Test	Test	Adequate
Environmental Fate			
Biodegradation	Test	Test	Adequate
Stability in Water	N/A	N/A	N/A
Photodegradation (estimate)	Adequate	Adequate	Adequate
Transport and Distribution	Adequate	Adequate	Adequate
Mammalian Toxicity			
Acute	Adequate	Adequate	Adequate
Repeat-dose	Adequate	Adequate	Test
Reprod/Develop	Test[1]	Test[1]	Test[1]
Genotoxicity, *in vitro*	Adequate	Adequate	Adequate
Genotoxicity, *in-vivo*	Adequate	Adequate	Adequate

[1] Data is adequate for Developmental endpoint

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APPENDIX A.

CAS Numbers and Definitions of Category Members

The CAS numbers and definitions of refinery streams, including gas oils and distillate fuels, were developed in response to Section 8(b) of the Toxic Substances Control Act. This section of TSCA required identification and registration with the Environmental Protection Agency before July 1979 of each "chemical substance" being manufactured, processed, imported or distributed in commerce. Due to analytical limitations and known variability in refinery stream composition, identification of every specific individual molecular compound in every refinery process stream under all processing conditions was impossible. Recognizing these problems, the American Petroleum Institute (API) recommended to the EPA a list of generic names for refinery streams consistent with industry operations and covering all known processes used by refiners. The list, including generic names, CAS numbers and definition of each stream, was published by the EPA as "Addendum I, Generic Terms Covering Petroleum Refinery Process Streams."

Because of the variability inherent in the processing of petroleum materials, the definitions API developed for the CAS numbers are qualitative in nature, written in broad, general terms. The definitions often contain only ranges of values for carbon numbers, with little if any quantitative analytical information or concern for possible compositional overlaps. As a result, the CAS descriptions are not useful in determining the exact composition of any specific refinery stream.

For example, included in Figure 3 are saturated hydrocarbons content values for samples of six gas oil streams with the CAS number 64741-49-7. The saturated hydrocarbon content of the six samples ranged from 49.1 to 67%. Thus, for the purposes of this test plan, one of the samples would be considered "predominantly aromatic" while the other five would be "predominantly saturated". It is because of this variability that the Testing Group has not grouped the CAS numbers of the gas oil streams listed in this appendix into "predominantly saturated" and "predominantly aromatic".

Distillate Fuels

068334-30-5

Diesel Oil ..C9-20 325F-675F
Petroleum products, diesel oil

A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 163 degrees C to 357 degrees C (325 degrees F to 675 degrees F).

068476-30-2

Fuel Oil No. 2 ..32.6 To 37.9 SSU

A distillate oil having a minimum viscosity of 32.6 SUS at 37.7 degrees C (100 degrees F) to a maximum of 37.9 SUS at 37.7 degrees C (100 degrees F).

068476-31-3

Fuel Oil No. 4 ..45 To 125 SSU

A distillate oil having a minimum viscosity of 45 SUS at 37.7 degrees C (100 degrees F) to a maximum of 125 SUS at 37.7 degrees C (100 degrees F).

068476-34-6

Diesel Fuel No. 2 ..32.6 To 40.1 SSU
Fuels diesel, no. 2

The distillate oil having a minimum viscosity of 32.6 SUS at 37.7 degrees C (100 degrees F) to a maximum of 40.1 SUS at 37.7 degrees C (100 degrees F).

Refinery Streams

064741-91-9

Solvent Refined Distillate, Middle..C9-20 302F-653F
Distillates (petroleum), solvent-refined middle

A complex combination of hydrocarbons obtained as the raffinate from a solvent extraction process. It consists predominantly of aliphatic hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 150 degrees C to 345 degrees C (302 degrees F to 653 degrees F).

06471-90-8

Solvent Refined Gas Oils..C11-25 401F-752F
Gas oils (petroleum), solvent refined

A complex combination of hydrocarbons obtained as the raffinate from a solvent extraction process. It consists predominantly of aliphatic hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205 degrees C to 400 degrees C (401 degrees F to 752 degrees F).

64741-44-2

Gas Oil, Light ..C11-20 401F-653F
Distillates (petroleum), straight- run middle

A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C20 and boiling in the range of 205 degrees C to 345 degrees C (401 degrees F to 653 degrees F).

06472-30-9

Neutralized Distillate, Middle ..C11-20 401F-653F
Distillates (petroleum) chemically neutralized middle

A complex combination of hydrocarbons produced by a treating process to remove acidic materials. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C20 and boiling in the range of approximately 205 degrees C to 345 degree C (401 degrees F to 653 degrees F).

06471-86-2

Sweetened Distillate ..C9-20 302F-653F
Distillates (petroleum), sweetened middle

A complex combination of hydrocarbons obtained by subjecting a petroleum distillate to a sweetening process to convert mercaptans or to remove acidic impurities. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 150 degrees C to 345 degrees C (302 degrees F to 653 degrees F).

06472-38-7

Clay Treated Distillate ..C9-20 302F-653F
Distillates (petroleum), clay-treated

A complex combination of hydrocarbons resulting from treatment of a petroleum fraction with natural or modified clay, usually in a percolation process to remove the trace amounts of polar compounds and impurities present. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 150 degrees C to 345 degrees C (302 degrees F to 653 degrees F).

64741-43-1

Gas Oil, Intermediate ..C11-25 401F-752F
Gas oils (petroleum), straight-run

A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205 degrees C to 400 degrees C (401 degrees F to 752 degrees F).

64741-49-7

Vacuum Tower Condensate ..C11-25 401F-752F
Condensates (petroleum), vacuum tower

A complex combination of hydrocarbons produced as the lowest boiling stream in the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205 degrees C to 400 degrees C (401 degrees F to 752 degrees F).

068915-97-9

Gas Oil, Heavy ..540F-660F
Gas oils (petroleum), straight-run, high-boiling

A complex combination of hydrocarbons produced by the atmospheric distillation of crude oil. It boils in the range of approximately 282 degrees C to 349 degrees C (540 degrees F to 660 degrees F).

06472-29-6

Neutralized Gas Oils..C13-25 446F-752F
Gas oils (petroleum), chemically neutralized

A complex combination of hydrocarbons produced by a treating process to remove acidic materials. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C25 and boiling in the range of approximately 230 degrees C to 400 degrees C (446 degrees F to 752 degrees F).

068814-87-9

Gas Oil, Intermediate ..C9-25 320F-752F
Distillates (petroleum), full-range straight-run middle

A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150 degrees C to 400 degrees C (302 degrees F to 752 degrees F).

64741-58-8

Vacuum Distillate, Light Paraffin ..C13-30 446F-842F
Gas Oils (petroleum), light vacuum

A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C30 and boiling in the range of approximately 230 degrees C to 450 degrees C (446 degrees F to 842 degrees F).

068915-96-8

Gas Oil Heavy ..550F-880F
Distillates (petroleum), straight-run, b. 557-880 degrees F.

06472-87-6

Hydrodesulfurized Gas Oil, Light Vacuum ..C13-30 446F-842F
Gas oils (petroleum), hydrodesulfurized light vacuum

A complex combination of hydrocarbons obtained from a catalytic hydrodesulfurization process. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C30 and boiling in the range of approximately 230 degrees C to 450 degrees C (446 degrees F to 842 degrees F).

06472-79-6

Hydrodesulfurized Gas Oil ..C13-25 446F-752F
Gas oils (petroleum), hydrodesulfurized

A complex combination of hydrocarbons obtained from a petroleum stock by treating with hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists predominantly of hydrocarbons having carbon numbers predominantly in the range of C13 through C25 and boiling in the range of approximately 230 degrees C to 400 degrees C (446 degrees F to 752 degrees F).

06472-46-7

Hydrotreated Distillate, Middle ..C11-25 401F-752F
Distillates (petroleum), hydrotreated middle

A complex combination of hydrocarbons obtained by treating a petroleum fraction with hydrogen in the presence of a catalyst. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205 degrees C to 400 degrees C (401 degrees F to 752 degrees F).

06472-80-9

Hydrodesulfurized Distillate, Middle ..C11-25 401F-752F
Distillates (petroleum), hydrodesulfurized middle

A complex combination of hydrocarbons obtained from a petroleum stock by treating with hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205 degrees C to 400 degrees C (401 degrees F to 752 degrees F).

06471-77-1

Hydrocracked Distillate, Light ..C10-18 320F-608F
Light Hydrocracked Distillate (Petroleum)

A complex combination of hydrocarbons from distillation of the products from a hydrocracking process. It consists predominantly of saturated hydrocarbons having carbon numbers predominantly in the range of C10 through C18, and boiling in the range of approximately 160 degrees C to 320 degrees C (320 degrees F to 608 degrees F).

068333-25-5

Hydrodesulfurized Distillate, Light Cat Cracked ..C9-25 302F-752F
Distillates (petroleum), hydrodesulfurized light catalytic cracked

A complex combination of hydrorcarbons obtained by treating light catalytic cracked distillates with hydrogen to convert organic sulfur to hydrogen sulfide which is removed. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150 degrees C to 400 degrees C (302 degrees F to 752 degrees F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons.

06471-60-2

Cat Cracked Distillate, Intermediate ..C11-30 401F-842F
Distillates (petroleum),intermediate catalytic cracked

A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C 30 and boiling in the range of approximately 205 degrees C to 450 degrees C (401degrees F to 842 degrees F). It contains a relatively large proportion of tricyclic aromatic hydrocarbons.

64741-59-9

Cat Cracked Distillate, Light ..C9-25 302F-752F
Distillates (petroleum), light catalytic cracked

A complex combination of hydrocarbons produced by the distillation of products from a catalytic cracking process. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C25 and boiling in the range of approximately 150 degrees C to 400 degrees C (302 degrees F to 752 degrees F). It contains a relatively large proportion of bicyclic aromatic hydrocarbons.

068333-88-0

Aromatic Hydrocarbons, C9-17

No description

068477-31-6

Reformate Still Bottoms, Light ..To 550F
Distillates (petroleum), catalytic, reformer fractionator residue, low-boiling

The complex combination of hydrocarbons from the distillation of catalytic reformer fractionator residue. It boils approximately below 288 degrees C (550 degrees F).

06471-82-8

Thermocracked Distillate, Light ..C10-18 320F-698F
Distillates (petroleum), light thermal cracked

A complex combination of hydrocarbons from the distillation of the products from a thermal cracking process. It consists predominantly of unsaturated hydrocarbons having carbon numbers predominantly in the range of C10 through C22 and boiling in the range of approximately 160 degrees C to 370 degrees C (320 degrees F to 698 degrees F).

Appendix B.

Links to Additional Resources

Refining Processes: General Descriptions

http://www.chevron.com/about/learning_center/refinery
http://www.lubrizol.com/lubetheory/default.htm
http://www.orionrefining.com/flow.htm
http://www.osha-slc.gov/dts/osta/otm/otm_toc.html
http://www.shellglobalsolutions.com/base_oils/library/library.htm
http://www.shell-lubricants.com/learningcenter/aboutoil.html
http://www.shellus.com/welcome/history/hist_oil_main.html
http://www.epa.gov/compliance/resources/publications/assistance/sectors/notebooks/petrefsnpt1.pdf
http://www.mts.net/~dbrad1/base_oil.htm

Petroleum Related Glossaries

http://www.caltex.com.au/products_glo.asp
http://www.citgo.com/CommunityInvolvement/Classroom/Glossary.jsp
http://www.epplp.com/gloss.html
http://www.prod.exxon.com/exxon_productdata/lube_encyclopedia/
http://www.hellenic-petroleum.gr/english/glossary/gl_main.htm
http://www.prod.exxon.com/exxon_productdata/lube_encyclopedia/
http://www.oilanalysis.com/dictionary
http://www.orionrefining.com/glossary.htm
http://www.gedolbear.com/glossary.htm
http://www.shellglobalsolutions.com/base_oils/glossary/a_g.htm
http://www.ursa-texaco.com/English/glossary_a.html
http://www.eia.doe.gov/pub/oil_gas/petroleum/data_publications/petroleum_marketing_annual/current/pdf/glossary.pdf

Appendix C.

Robust Summary

(Separate document)

ROBUST SUMMARY
OF INFORMATION ON

Substance Group # Gas oils

Summary prepared by American Petroleum Institute

Creation date: February 27, 2002

Printing date: November 6, 2003

Date of last Update: **November 3, 2003**

Number of pages: **65**

NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : Gas oils are complex mixtures of hydrocarbons that boil over the range 150 to 450 °C and with carbon numbers predominantly in the range C9 to C30.

Gas oils contain straight and branched chain alkanes, cycloalkanes, aromatic hydrocarbons and mixed aromatic cycloalkanes. Olefins will also be present in gas oils that have been produced from a cracking process but in general are only present in small quantities.
Some gas oils (straight run materials) contain 2- and 3-ring PACs with relatively low concentrations of 4- to 6-ring PACs. Whereas heavier atmospheric, vacuum or cracked gas oil streams will contain higher levels of the 4- to 6-ring PACs.

Mammalian and geno- toxicity studies have been conducted on a number of gas oil samples. All of the samples have been characterized in detail and this information has been published as follows:

API samples	(API 1987)
DGMK Samples	(DGMK 1991)
CONCAWE samples	(CONCAWE 91/51)
MOBIL samples	(Feuston et al, 1994)
ARCO samples	(In relevant study report)

Although the CAS numbers may not have been included in some of the above reports the samples were all described in a generic sense and related to their method of manufacture e.g. straight run or cracked gas oil. Furthermore, all the reports clearly identified the samples as gas/fuel oils, and provided sufficient information to allow the test materials to be assigned to the appropriate grouping, "predominantly saturates" or "predominantly aromatics".

Of particular importance is the information on hydrocarbon types and this information is tabulated below. The samples have been subdivided into those that contain predominantly saturates or predominantly aromatics.

Sample (CAS No.)	**Saturates**	**Aromatics**	**Olefins**
Predominantly Aromatic			
MOBIL LCO* (64741-59-9)	20.2	79.8	0
API 83-07 (64741-59-9)	24	72.4	3.7
CONCAWE MD7 (64741-59-9)	29.1	69.1	1.8
API 83-08 (64741-59-9)	31.4	60.8	7.8
DGMK 10	40.2	59.8	0
DGMK 7	44.9	55.1	0
DGMK 14	47.6	52.4	0
DGMK 6	47.9	48.3	3.8

Predominantly saturates			
DGMK 13	52.5	46.9	0.6
DGMK 12	56.1	42.6	1.3
MOBIL CLGO* (64741-82-8)			
	56.9	43.1	0
DGMK 9	57.5	42.5	0
DGMK 2	65.3	32.6	2.1
ARCO F-215	65.4	34.6	0
API 81-10 (64742-80-9)			
	65.6	34.4	0
DGMK 5	66.1	33.9	0
DGMK 4	70.7	29.3	0
DGMK 3	71.9	28.1	0
DGMK 8	73	27	0
CONCAWE MD6 (64742-46-7)			
	73.8	22.6	3.6
DGMK 11	74.6	21	4.4
DGMK 1	76.4	23.6	0
ARCO F-220	77.7	22.3	0
API 83-11 (64741-44-2)			
	78.8	21.2	0
API 81-09 (64742-80-9)			
	79.4	20.6	0
ARCO F-188	86	14	0

* LCO = Light cycle oil
CLGO = Coker light gas oil

For most of the mammalian toxicology endpoints, information has been used that was derived by the American Petroleum Institute on a wide range of gas oil streams. For simplicity, this robust summary contains detailed information on a single API sample for each endpoint and if data were available on other samples for the same endpoint they have been summarized in tabular form in the relevant sections or discussed in detail when appropriate.

(30) (43) (52)

1.13 REVIEWS

Memo : CONCAWE

Remark : CONCAWE has summarized the available health effects data and also data on environmental effects in a non-critical review.

(44)

Memo : ATSDR

Remark : ATSDR published a toxicology review on fuel oils.

(40)

Memo : IARC

Remark : IARC reviewed the available data on distillate fuels and assessed the strength of evidence that the fuels were a carcinogenic risk to man and animals.

The conclusions of the IARC review were:

Evaluation:
There is inadequate evidence for the carcinogenicity in humans of diesel fuels.

There is limited evidence for the carcinogenicity in experimental animals of marine diesel fuel.

There is limited evidence for the carcinogenicity in experimental animals of fuel oil No. 2.

The overall evaluations were:

Marine diesel fuel is possibly carcinogenic to humans (Group 2B)

Distillate (light) diesel fuels are not classifiable as to their carcinogenicity to humans (Group 3)

Distillate (light) fuel oils are not classifiable as to their carcinogenicity to humans (Group 3)

(54)

2.1 MELTING POINT

Method : ASTM D97
GLP : No data

Remark : Melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 1999). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 1999). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e., crystal formation) and/or viscosity property.

The following pour point data reflect characteristic values for high aromatic gas oils. Values typical for high saturate gas oils will be similar to those of the distillate fuels, which generally fall into the high saturate subcategory. However, the pour point values of some distillate fuels may be lower than a corresponding gas oil due to the addition of additives designed to lower the fuel's pour point.

Result : Light Cat-Cracked Distillate
(CAS No 64741-59-9)

Sample	Pour point (°C)	Method	Ref
API 83-07	-12	ASTM D97	API 1987
API 83-08	-15	ASTM D97	API 1987

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier and reference database.

(30) (38)

2.2 BOILING POINT

Method : ASTM D86
GLP : No data

Result :

Sample	Boiling Range °C	Method	Ref.
Predominantly aromatics			
Light Cat-Cracked Distillate (CAS No 64741-59-9)			
API 83-07	240-372	ASTM D86	API 1987
API 83-08	185-351	ASTM D86	API 1987

Predominantly saturates
Hydrodesulfurized Gas Oil (CAS No. 64742-80-9)

API 81-09	261-301	ASTM D86	API 1987
API 81-10	172-344	ASTM D86	API 1987

Straight-Run Gas Oil (CAS No. 64741-44-2)

API 83-11	185-391	ASTM D86	API 1987

Reliability : (2) valid with restrictions
Results of standard method testing were reported in a reliable review dossier and a reference database.

(30)

2.4 VAPOUR PRESSURE

Value : at 25 °C
Method : Estimated (EPIWIN, MPBPWIN V1.40; U.S. EPA 2000)
GLP : N

Remark : It is predicted from vapor pressure modeling that C9 to C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon components of gas oils will have approximate vapor pressure values <2 kPa at 25 °C. As hydrocarbon chain lengths exceed C15, vapor pressures fall below levels capable of being measured by standardized techniques (OECD Guideline 104, Vapor Pressure; OECD 1995). The vapor pressure of complex mixtures is equal to the sum of the vapor pressures of the individual constituents in their pure form times their mole fraction in the mixture (Raoult's Law). Therefore, the total vapor pressure of a gas oil will depend on the proportion of different molecular weight constituents making up the mixture. These estimated vapor pressures of component hydrocarbons in gas oils are generally within the range and support the measured values for distillate fuels.

Result :

Vapor Pressure Estimates (kPa)

	Number of C Atoms		
	C9	**C15**	**C30**
Paraffins			
n-	0.6	$5x10^{-4}$	$5x10^{-8}$
iso-	1.3	$6x10^{-3}$	$2x10^{-8}$
Naphthenes			
1-ring	0.6	$3x10^{-4}$	$6x10^{-9}$
2-ring	0.3	$3x10^{-3}$	$7x10^{-9}$
Olefins			
straight	0.7	$6x10^{-4}$	$9x10^{-9}$
cyclic	0.4	$2x10^{-3}$	$6x10^{-9}$
Aromatics			
1-ring	0.5	$8x10^{-4}$	$9x10^{-10}$
2-ring	0.01 (1)	$5x10^{-5}$	$1x10^{-10}$

(1) 10 carbon atoms in structure

Reliability : (2) valid with restrictions
Estimations made using a validated computer model

(66)

2.5 PARTITION COEFFICIENT

Partition coefficient : Octanol-water
Method : Calculated by KOWWIN, V 1.66 (EPIWIN V 3.10; EPA 2001)
Year : 2001
GLP : No

Remark : Kow values of representative C9 and C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon components of gas oils were modeled. From the carbon number range of modeled individual hydrocarbon structures, the apparent range of Kow values of these substances will extend from 3.3 to >6 (API 1987).

The modeled values given above are consistent with the Kow estimates of 3.9 to >6 calculated by CONCAWE (1996) based on known hydrocarbon composition of a hydrodesulfurized gas oil (CAS No. 64742-80-9), a straight-run gas oil (CAS No. 64741-44-2) and a light catalytic cracked gas oil (CAS No. 64741-59-9). These Kow estimates cover gas oil streams having both high saturate and high aromatic fractions.

Result :

Log Kow = 3.3 to approximately 13

	Log Kow values No. of C atoms	
	C9	**C30**
Paraffins		
n-	4.8	16
iso-	4.7	15
Naphthenes		
1-ring	4.6	15
2-ring	3.7	14
Olefins		
straight 5.2	15	
cyclic	4.5	13
Aromatics		
1-ring	3.7	14
2-ring	3.3*	13

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(30) (44) (45) (64)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : < 0 .001 mg/l at 25 °C
Method : Calculations by WSKOW V 1.40. (EPIWIN V 3.10; EPA 2001
Year : 2001
GLP : No

Remark : It is predicted from solubility modeling that C9 to C30 paraffinic,

naphthenic, olefinic and aromatic hydrocarbon components of gas oils will have approximate water solubility values of 0.1 to approximately 52 mg/l at 25 °C. As hydrocarbon chain lengths exceed C15, these compounds become increasingly hydrophobic to the point where solubility becomes negligible. Hydrocarbon components of gas oils in the range C16 to C30 typically have water solubility values less than 0.1 mg/l. The ultimate solubility of a specific gas oil will depend not only on the relative percentage of aromatic versus saturate fractions but also on the molecular weights of those principal hydrocarbons.

Result :

Water solubility estimates (mg/l)

	No. of C atoms C9	**C15**	**C30**
Paraffins			
n-	2.3	<0.001	<0.001
iso-	2.7	<0.001	<0.001
Naphthenes			
1-ring	3.4	0.004	<0.001
2-ring	19	0.26	<0.001
Olefins			
straight 1.1	0.004	<0.001	
cyclic	4.1	0.005	<0.001
Aromatics			
1-ring	52	0.035	<0.001
2-ring	31*	0.63	<0.001

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(65)

3.1.1 PHOTODEGRADATION

Type : Calculated
Method : Calculated: by AOPWIN V 1.90 (EPIWIN V 3.10; EPA 2001
Year : 2001
GLP : No
Test substance : TS: Gas oils, various

Remark : Direct photolysis is not expected to be a major degradation pathway for most of the components in gas oils. Chemicals having the potential to photolyze have UV/visible absorption maxima in the range of 290 to 800 nm. Most hydrocarbon constituents in this category are not expected to photolyze since they do not show absorbance within the 290-800 range. However, where unsaturated hydrocarbons, notably aromatic hydrocarbons are present in, or near the surface of water, degradation by reaction with sunlight in the presence of oxygen can be a significant removal process (CONCAWE 2001).

It is predicted from indirect photolysis modeling of C9 to C30 paraffinic, naphthenic, olefinic and aromatic hydrocarbon compounds that volatile components in gas oils will undergo moderate atmospheric oxidation and not persist in the environment. Base on the modeled half-life values of component hydrocarbon structures, gas oils containing primarily aromatic or saturate fractions are not expected to show significant differences in their photodegradation characteristics.

Result : Direct photolysis:
Most substances in this category are not subject to direct photolysis; see remarks section below.

Half-life	N/A
Degradation	N/A
Quantum yield	N/A

Indirect photolysis:

Sensitizer type	Hydroxyl radicals (OH-)
Conc. of sensitizer	1.5×10^6 OH^-/cm^3
Rate constant	various
Half-life	see table of half-lives below (given in days)
Breakdown products	N/A

	Half life values (days) No. of C atoms		
	C9	**C15**	**C30**
Paraffins			
n-	1.1	0.6	0.3
iso-	1.1	0.6	0.3
Naphthenes			
1-ring	0.8	0.5	0.2
2-ring	0.8	0.4	0.2
Olefins			
straight	0.3	0.2	0.2
cyclic	0.1	0.1	0.1
Aromatics			
1-ring	1.5	0.7	0.3

2-ring	0.5*	0.2	0.1

* Value given for a C10 molecule

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(45) (63)

3.1.2 STABILITY IN WATER

GLP : No

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components found in the materials that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

Reliability : (1) valid without restriction
(53)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated

Method : Calculations by fugacity-based environmental equilibrium partitioning model (EQC model)

Remark : Multimedia distribution was calculated for low and high molecular weight hydrocarbon compounds representing common PONA (i.e., paraffinic, olefinic, naphthenic and aromatic) constituents in gas oil streams. Partitioning behavior depends largely on molecular weight, with smaller compounds (e.g., 9 to 15 carbon atoms) partitioning to the air due to relatively high vapor pressures. Here they are expected to degrade rapidly via hydroxyl radical attack (indirect photodegradation). Larger molecular weight hydrocarbons (e.g., 15 to 30 carbon atoms) partition to the terrestrial environment where they are expected to undergo slow biodegradation. Mobility in the aquatic environment is low due to low water solubility or to high vapor pressure in those compounds showing appreciable water solubility limits. Based on the relative distribution of component hydrocarbons, gas oils having principally aromatic or saturate fractions are not expected to show significant differences in environmental partitioning.

Result : Media:
Air, Water, Soil, Sediment, Suspended Sediment, Fish.

PERCENT DISTRIBUTION

	Air	Water	Soil	Sed.	Susp. Sed.	Fish
n-Paraffins						
C9	99	<0.1	1	<0.1	<0.1	<0.1
C15	13	<0.1	85	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1

Iso-paraffins						
C9	99	<0.1	0.5	<0.1	<0.1	<0.1
C15	68	<0.1	31	0.7	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
Straight olefins						
C9	99	<0.1	0.7	<0.1	<0.1	<0.1
C15	17	<0.1	81	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
Cyclic olefins						
C9	99	0.3	0.7	<0.1	<0.1	<0.1
C15	49	<0.1	50	1	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
1-ring naphthenes						
C9	99	<0.1	0.9	<0.1	<0.1	<0.1
C15	0.4	<0.1	97	2	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
2-ring naphthenes						
C9	99	0.2	1	<0.1	<0.1	<0.1
C15	51	<0.1	48	1	<0.1	<0.1
C30	0.1	<0.1	98	2	<0.1	<0.1
1-ring aromatics						
C9	97	1	2	<0.1	<0.1	<0.1
C15	19	<0.1	79	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1
2-ring aromatics						
C10	77	8	15	0.3	<0.1	<0.1
C15	0.7	0.2	97	2	<0.1	<0.1
C30	<0.1	<0.1	98	2	<0.1	<0.1

(55)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Adapted inoculum of domestic activated sludge
Contact time : 28 day(s)
Method : CONCAWE. Test method for determining the inherent aerobic biodegradability of oil products. 1996/1997, and modification of ISO/DIS 14593
Year : 1993
GLP : No
Test substance : Gas oils (petroleum), solvent-refined; CAS No. 64741-90-8.

Method : Water quality-Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium-Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
Result : Test material was inherently biodegradable since it achieved >20% biodegradability based on CO_2 production.

	% Degradation (sd)	
Test Day	**Aniline**	**Gas oil**
7	82.98 (1.018)	35.85 (0.636)
14	78.19 (0.575)	41.96 (2.517)
21		39.40 (0.926)
28		34.82 (0.156)*

*Lower values due to recalibration using standard concentration exceeding linear range of quantitation.

Source : American Petroleum Institute.

Test condition : Acclimated inoculum prepared from activated sludge was incubated with test substance and aniline (positive control) during a two week adaptation period in separate test systems. Test medium consisted of 1 liter glass distilled water and mineral salts (10 ml/l phosphate buffer, 1ml 0.025% ferric chloride, 1ml 2.75 magnesium sulfate, and 1 ml 2.75% calcium chloride solutions) prepared as described in ISO method.

Acclimation of inoculum was performed using activated sludge from the aeration basin of Somerset-Raritan Valley Wastewater Treatment Plant (Bridgeport, NJ., U.S.A.). On the day of collection, the sludge was returned to the laboratory and homogenized for two minutes in a blender at medium speed. The supernatant was removed from the homogenated sample after a settling period of at least 30 minutes and then filtered through Whatman #4 coarse filter paper. One hundred mls of the filtered sludge was combined with 900 ml test medium in a 2 liter flask for each of the two acclimation test systems. Gas oil or aniline were administered to the appropriate flask on days 0, 7 and 11 to achieve approximately 4, 8 and 8 mg of carbon as test or positive control substance. The flasks walls were covered to prevent light exposure to the inoculum, loosely stoppered with gauze to allow aeration and placed on a gyratory shaker set at 200 rpm. An aqueous stock solution of aniline was used to dose the positive control acclimating inoculum. The gas oil was introduced as neat material using a tared 10 µl Hamilton glass syringe, and amounts added were determined on a gravimetric basis.

On day 14 of incubation gas oil acclimated inoculum was transferred to a separatory funnel and approximately half the volume of aqueous phase was drained from the funnel for testing, avoiding any carryover of residual gas oil. The drained gas oil inoculum was combined with an equal volume of the aniline acclimated inoculum and mixed thoroughly. One hundred ml of the composite inoculum was mixed with 900 ml test medium, and then used to prepare the biodegradation test systems.

Biodegradation test vessels (125 ml serum bottles) were filled with 100 ml of the inoculated test medium. Blank test systems received no additional treatment and were sealed immediately. Addition of the respective substances was performed as described for the acclimation procedure to achieve test concentrations of approximately 10 mg carbon/l. Duplicate test systems for gas oil, aniline and blank treatments were prepared for sacrifice at weekly sampling intervals for subsequent CO_2 analysis. All samples not designated for time zero analysis were crimp sealed, covered with aluminum foil and agitated on a gyratory shaker at approximately 200 rpm. CO_2 determination was performed on days 0, 7, 14, 21 and 28. At each sampling interval, the appropriate test systems were basified with 0.5 ml of 10N NaOH (pH>12) in order to convert CO_2 in the headspace to water soluble sodium carbonate, returned to the gyratory shaker for an

hour and then analyzed. Total inorganic carbon (TIC) in the aqueous phase was measured using an O.I. Model 700 carbon analyzer calibrated with sodium carbonate standards. On days 0, 7, 14 and 21 standard concentrations used were suitable to quantify up to 25 ppm carbon, on day 28 calibration curve was developed to quantify up to 50 ppm carbon.

Reliability : (2) valid with restrictions
The data reported in this report were obtained through participation with CONCAWE task group for inherent biodegradation method development of water insoluble substances. The gas oil sample was supplied by Burmah Castrol as part of research project to identify procedures for inherent biodegradation test Thus, although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs.

(51)

5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}
Value : 6790 - 7180 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Vehicle : None
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : The test material was administered undiluted, as a single oral dose to groups of 5 male at each of 5 dose levels and 5 female rats at each of 3 dose levels. The rats had been fasted for 24 hours prior to dosing but had free access to water.
Following dosing, food and water were available ad-lib for a period of 14 days. The animals were observed for clinical signs of toxicity and mortality every hour for the first 6 hours after dosing and twice daily thereafter for 14 days. The rats were weighed just prior to dosing and then at 7 and 14 days after dosing.
At study termination all animals were killed with carbon dioxide and subjected to a gross necropsy and abnormalities were recorded.
The LD_{50} and 95% confidence limits were calculated using a standard technique.

Result : Clinical signs seen during the study included: hypoactivity, diarrhea, yellow-stained urogenital/abdominal area, hair loss on anal region/abdomen/hind legs, ataxia, red-stained nose and mouth, prostration, lacrimation, catalepsy, dyspnea, possible respiratory congestion, hypothermic to touch, inflamed anal region and death.
Mortalities were as follows:

Dose (g/kg)	No. dead/ No. dosed
Males	
5.0	0/5
6.25	3/5
7.81	3/5
9.76	3/5
12.2	5/5
Females	
5.0	1/5
6.25	1/5
7.81	4/5

Reliability : (1) valid without restriction

(18)

Type : LD_{50}
Species : Rat
Test substance : Gas oils various. See section 1.1.1

Result : In addition to the study summarized above, the American Petroleum Institute reported acute oral toxicity studies for four other gas oils. In all five studies the clinical signs were similar irrespective of the test material.

All studies are considered to be reliability 1 (Klimisch) and therefore only one study has been described fully in the robust summary above.

The following table summarizes the LD_{50} data available for all five studies (including API 83-08) that have been reported.

Sample	CAS No.	Oral LD_{50} (g/kg)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	M 4.66 F 3.2	API 33-30162
API 83-08	64741-59-9	M 7.18 F 6.79	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	>5.0	API 30-32348
API 83-11	64742-44-2	>5.0	API 32-32857
API 81-09	64742-80-9	>5.0	API 30-32347

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC_{50}
Species : Rat
Strain : Sprague-Dawley
Sex : Male/female
Number of animals : 5
Exposure time : 4 hour(s)
Year : 1987
GLP : Yes
Test substance : API 83-11, predominantly saturates, see section 1.1.1.

Method : 5 male and 5 female rats were exposed to 6 different concentrations of test substance by whole body exposure for 4 hours. The study was conducted in two parts. The first part consisted of exposures at a single nominal concentration of 5 mg/l, the second part consisted of exposures at nominal concentrations of 0, 3.3, 4.78, 6.55 and 7.58 mg/l. After exposure for 4 hours, the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia. For all animals, including those found dead during the study the lungs and any grossly abnormal tissues were removed, fixed and examined histologically.

Result : Results of chamber monitoring and overall mortalities are shown in the following table

Chamber concentration (mg/l)		No. Dead/No. exposed	
Nominal	Actual	Male	Female
14.1	5.39	5/5	5/5
0.0	0.01	0/5	0/5
3.31	1.05	0/5	0/5
6.55	3.22	4/5	5/5
4.78	1.6	3/5	1/5
7.58	2.25	4/5	5/5

The actual concentrations were used to determine the LC_{50} values. These were as follows:

Sex	LC_{50} (mg/l)	95% Confidence limits (mg/l)
Male	1.72	1.22 to 2.42
Female	1.82	1.45 to 2.28
Male & Female	1.78	1.44 to 2.2

There was an apparent dose-related weight suppression at 7 days but at 14 days body weight gains were observed in surviving animals. Clinical signs during the exposure consisted of: decreased activity, wet inguinal area, eyes partially closed, wet coat and oily coat. In the seven days following exposure there were signs of poor condition and respiratory distress. In the second week survivors were considered to be normal in appearance.
Gross post mortem findings included oily hair coat which was attributable to deposition of test material. Additionally, dark red lungs were observed in all animals that died within a day or two of exposure. This was not observed in any animal that survived the exposure.
A diffuse moderate or marked pulmonary congestion and perivascular edema was seen in all animals that died during the study. Spotty alveolar edema was also seen but less consistently.
In all animals that had died during the study widespread damage had occurred to the alveolar walls resulting in fibronecrotic debris and extravasation of RBCs and PMNs. Necrosis was seen in the walls of small blood vessels and there was spotty epithelial necrosis in small bronchioles, but the most severe damage seemed to be centroacinar. Larger airways were relatively unaffected. Animals surviving to term did not exhibit the above changes but survivors that had been exposed to levels of 1.5 mg/l and above exhibited chronic inflammatory changes in the lungs.

Test condition : Exposures were conducted in stainless steel and glass chambers of 0.25 cubic meter volume. The rats were individually housed during exposure. Aerosols of API 83-11 were generated using a nebulizer and were introduced to the exposure chamber intake where it was diluted with room air to achieve the target concentration. Actual chamber concentrations were determined gravimetrically by collection of aerosols on filters.

Reliability : (1) valid without restriction

(1)

Type : LC_{50}
Test substance : Gas oils various, see section 1.1.1.

Result : The American Petroleum Institute have reported four other acute inhalation toxicity studies on gas oil samples.

The LC_{50} determined in all studies (including the one summarized above) are as follows:

Sample	CAS	LC_{50} (mg/l)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	5.4*	API 33-30549
API 83-08	64741-59-9	4.65**	API 33-30444

Predominantly saturates			
API 81-10	64742-80-9	7.64	API 30-32857
API 83-11	64742-44-2	M 1.72 F 1.82 M&F 1.78	API 34-3065
API 81-09	64742-80-9	4.60	API 30-32856

* LC_{50} 5.4 mg/l for combined sexes.
3.35 mg/l for males; female data inadequate to calculate female LC_{50} alone.

** No deaths occurred during the exposure period at 5.06 mg/l aerosol. However, 3 males and 1 female from this group died during the 14-day observation period. (Repeated at 2.34 - 7.29 mg/l) LC_{50} of 4.65 mg/l for combined sexes but data insufficient for determination of LC_{50} for females alone.

Reliability : (1) valid without restriction

(8) (9) (25) (26)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 8
Year : 1985
GLP : Yes
Test substance : TS: API 83-08, predominantly aromatic, see section 1.1.1.

Method : A weighed quantity of undiluted test material (equivalent to a dose of 2 g/kg) was applied to the dorsal skin of each of 4 male and 4 female rabbits. The skin of the patched area of two rabbits of each sex had been abraded whilst the other two had intact skin. The applied dose was covered with an occlusive dressing (gauze and an impermeable covering). 24 hours after dosing, the patches were removed, the skin wiped and collars fitted to the rabbits to prevent oral intake of any residual test material. The collars were removed 24 hours later and the animals were observed for a total of 14 days post-dosing.
At study termination the animals were killed with carbon dioxide and a gross necropsy was performed. Any abnormalities were recorded.

Result : No signs of systemic toxicity or death occurred at the dose level of 2 g/kg in this study.
Dermal irritation ranged from slight to severe for erythema and edema, from slight to moderate for atonia, desquamation and coriaceousness, and from slight to marked for fissuring. Other dermal irritation seen included subcutaneous hemorrhage and blanching.

Conclusion : The LD_{50} was greater than 2 g/Kg in both sexes for both intact and abraded skin.

Reliability : (1) valid without restriction

(18)

Type : LD_{50}
Test substance : Gas oils various, see section 1.1.1.

Result : The American Petroleum Institute reported five acute dermal toxicity studies for gas oils including sample API 83-08 (described above).
In all studies the clinical signs were similar irrespective of the test material.
All studies are considered to be reliability 1 (Klimisch) and therefore only one study has been described fully in the robust summary above.

The following table summarizes the LD_{50} data available for all five studies (including API 83-08) that have been reported.

Sample	CAS No.	Dermal LD_{50} (g/kg)	Reference
Predominantly aromatic			
API 83-07	64741-59-9	>2.0	API 33-30162
API 83-08	64741-59-9	>2.0	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	>2.0	API 30-32348
API 83-11	64742-44-2	>2.0	API 32-32857
API 81-09	64742-80-9	>2.0	API 30-32347

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : 6.9
Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.5 ml of undiluted test material was applied to two areas on each of six rabbits. One area was intact and the other abraded skin. The treated area was then covered with an occlusive dressing.
After 24 hours the dressing was removed and the treated skin was wiped to remove any residue of test material. The degree of erythema and edema was recorded according to the Draize scale. A second reading of skin responses was made at 72 hours again at 96 hours, 7 and 14 days.
Results of the 24 and 72 hour readings were used to determine the Primary Irritation Index.

Result : Moderate to severe levels of irritation occurred in this study.
There was no real difference in the severity of the response on either intact or abraded skin. Average irritation scores were as follows for abraded skin:

	Erythema	Edema
24 hours	3.2	3.2
72 hours	3.7	3.7
96 hours	3.5	3.3
7 days	2.2	1.7
14 days	0.3	0.0

Blanching was seen in 2 animals at 24 hours and in six animals at 72 hours. At 96 hours subcutaneous hemorrhaging within the test sites was

observed in all animals A possible necrotic area was seen in one animal at 96 hours.

Reliability : (1) valid without restriction

(18)

Species : Rabbit
Test substance : Gas oils various, see section 1.1.1.

Result : Seven skin irritation studies have been reported for gas oil samples, including sample API 83-08. All the available data (including that for API 83-08) are summarized below.

It is interesting to note that the predominantly aromatic gas oils were slightly more irritant than the predominantly saturate gas oils.

Sample	CAS No.	Irritation index	Reference
Predominantly aromatic			
API 83-07	64741-59-9	5.6	API 33-30162
CONCAWE MD 7	64741-59-9	*	CONCAWE 91/51
API 83-08	64741-59-9	6.9	API 32-32859
Predominantly saturates			
API 81-10	64742-80-9	5.9	API 30-32348
CONCAWE MD 6	64742-46-7	**	CONCAWE 91/51
API 83-11	64742-44-2	3.2	API 32-32857
API 81-09	64742-80-9	4.3	API 30-32347

Samples MD 6 and MD 7 were tested in a 4 hour semi-occlusive patch test in rabbits.

* MD-7 elicited well defined erythema in one animal and moderate/severe erythema in two animals at the 60 minute, Day 1, Day 2, and Day 3 observations. One animal had well defined erythema on Day 7. Edema, which ranged from very slight to moderate, was observed in all animals up till the 72 hour stage. Desquamation was seen in all animals at day 7 and in only one animal at day 10. At day 10 all animals were free from erythema and/or edema.

** MD-6 elicited minimal, transient dermal irritation in all 3 animals. At the 60 minute observation time, well-defined erythema and very slight edema was observed. Very slight erythema was observed in all animals at day 1, 2 animals on days 2 and 3, and one animal on day 7. Two animals were observed with desquamation on day 7. All animals were free of dermal abnormalities at the 10 day observation.

Reliability : (1) valid without restriction

(2) (4) (5) (19) (48) (50)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Number of animals : 9

Method : Draize Test
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.1 ml of undiluted test material was applied to the corneal surface of one eye of each of 9 rabbits, the other eye was untreated and served as control. After 30 seconds the treated eyes of 3 rabbits were washed with lukewarm water for 1 minute. Eyes of the other 6 rabbits were not washed. Readings of ocular lesions for all animals were made at 1, 24, 48, 72 hours and 7 days after treatment. Sodium fluorescein was used to aid in revealing possible corneal injury.

Result : There was no pain response from any animal following instillation of the test material onto the cornea. No corneal irritation was seen during the study. However, a white fibrin filament was seen in the anterior chamber in one animal (unwashed eye group) at 48 and 72 hours.

Primary eye irritation scores recorded in this study are as follows:

	1 Hr.	24 Hrs	48 Hrs.	72 Hrs.	7 days
Unwashed eyes	4.3	3.2	2.2	1.2	0.0
Washed eyes	4.7	0.0	0.0	0.0	0.0

Reliability : (1) valid without restriction

(18)

Species : Rabbit
Test substance : Gas oils various, see section 1.1.1.

Result : Five eye irritation studies have been reported for gas oil samples, including sample API 83-08. The irritation scores at 24 hours (including those for API 83-08) are tabulated below.
The predominantly aromatic materials appear to be slightly more irritating than the predominantly saturate gas oils.

Sample	**CAS No.**	**Eye Irritation Index**		**Reference**
		rinsed eye	**Unrinsed eye**	
Predominantly aromatic				
API 83-07	64741-59-9	2.0	1.7	API 33-30162
API 83-08	64741-59-9	0.0	3.2	API 32-32859
Predominantly saturates				
API 81-10**	64742-80-9	0	1.0	API 30-32348
API 83-11	64742-44-2	0.0	1.0	API 32-32857
API 81-09*	64742-80-9	0	2.0	API 30-32347

* 1 hour after instillation of test material, the average eye irritation scores were 2.7 and 0.7 for the unrinsed and rinsed eyes respectively

** 1 hour after instillation of the test material, the average irritation index was 2.7 for both the rinsed and unrinsed eyes

*** average eye irritation index for 48 hour readings were 2.0 and 0.67 for unrinsed and rinsed eyes respectively

**** At 48 hours the average eye irritation index was 0 for both rinsed and unrinsed eyes

***** At 48 hours the average eye irritation index was 0 and 0.33 for rinsed and unrinsed eyes respectively

Reliability : (1) valid without restriction

(2) (4) (5) (19)

5.3 SENSITIZATION

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge 10 % occlusive epicutaneous
Number of animals : 76
Result : not sensitizing
Year : 1985
GLP : Yes
Test substance : API 83-08, predominantly aromatic, see section 1.1.1.

Method : 0.4 ml undiluted test material was applied under an occlusive dressing to the shaved skin of 10 male and 10 female animals. Six hours after application the dressing was removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same application site was used each time. Two weeks following the third application a challenge dose (0.4 ml of a 10% solution in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application.
The application sites for sensitizing and challenge doses were read for erythema and edema 24 and 48 hours after patch removal. To assist in the reading of the response to the final challenge dose the test site was depilated 3 hours prior to reading by using a commercially available depilatory cream.

Positive control, vehicle control and naive control groups were included in this study.

Concentrations of positive control were as follows:

Sensitizing doses: 0.4 ml of 0.3% w/v in 80% aqueous ethanol
Challenge dose: 0.4 ml of 0.1% w/v suspension in acetone

Result : A mixture of clinical signs were observed during the study but these were not confined to any single test group and were not related to the test material. The signs included soft stools/diarrhea, hypothermia, red ocular discharge and body weight loss. A total of 3 animals died.
Skin reactions following the challenge application were as follows:

Test group: Very slight erythema in 3/9 animals
Naive control: Very slight erythema in 3/9 animals
Vehicle control: Very slight erythema in 1/9 animals
Positive control: Very slight to severe irritation 20/20 animals. The reaction of 19 exceeded the highest reaction observed in the naive positive control group
Naive positive control: Very slight erythema in 5/20 animals

Reliability : (1) valid without restriction

(18)

Type	:	Buehler Test
Species	:	Guinea pig
GLP	:	Yes
Test substance	:	Gas oils various, see section 1.1.1.

Result : In addition to the test described above on sample API 83-08, four other samples of gas oil were tested and found to be non-sensitizing. References to all the appropriate studies are as follows:

Sample	CAS No.	Reference
Predominantly aromatic		
API 83-07	64741-59-9	API 33-30162
API 83-08	64741-59-9	API 32-32859
Predominantly saturates		
API 81-10	64742-80-9	API 31-31414
API 83-11	64742-44-2	API 32-32857
API 81-09	64742-80-9	API 31-31352

Reliability : (1) valid without restriction

(3) (10) (11) (18) (19)

5.4 REPEATED DOSE TOXICITY

Remark : Six 28-day dermal studies and two 90-day dermal studies have been reported for gas oils. Whereas one of the 28 day studies involved daily application of test material five days each week, the other 28 day studies involved application three times weekly.
Full robust summaries are given for both of the 90-day studies.
A full robust summary is given for the 28 day study in which test material was applied daily, five times per week.
A full robust summary is also given for a 28 day study in which test material was applied three times weekly.
A summary table of the results of all the studies involving thrice weekly application of test material is also provided in this section.

Species	:	Rabbit
Sex	:	Male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Exposure period	:	6 Hours
Frequency of treatm.	:	Once a day, three times weekly for 4 weeks
Doses	:	250, 500 & 1000 mg/kg
Control group	:	Yes, concurrent no treatment
Year	:	1985
GLP	:	Yes
Test substance	:	API 83-07 (predominantly aromatic) see section 1.1.1.

Method : Approximately 24 hours before administration of the test material, the dorsal skin was clipped free of hair. The exposure site was subsequently reclipped as necessary throughout the study. Immediately prior to the first application of test material, the skin was examined and scored for irritation using the standard Draize scoring procedure for erythema and edema.

Undiluted test material was applied to the skin of 5 male and 5 female rabbits at dose levels of 250, 500 and 1000 mg/kg. The males weighed between 2.7 and 3.2 kg and the females weighed between 2.6 and 3.3 kg at the start of the study. The treated site was covered with gauze and an occlusive dressing and these remained in place for a 6 hours exposure period. At the end of the exposure, the dressings were removed and any residual test material was removed by wiping with a dry gauze pad. Dosing was carried out 3 times weekly for 4 weeks i.e. until 12 doses had been applied. A group of 5 male and 5 female animals served as sham controls.
Checks were made twice daily for mortality, moribundity and for signs of pharmacologic and toxic effects. Body weights were recorded immediately before the first dose was applied and weekly thereafter. Before each application of test material the skin was examined and scored for erythema and edema as before. Animals that died during the study were subjected to a complete gross necropsy. At the end of the dosing period surviving animals were sacrificed and a complete gross necropsy was performed.
At termination, blood samples were taken from every animal and determinations were made of: RBC count, WBC count, differential WBC count, hemoglobin concentration, hematocrit, glucose, blood urea nitrogen, alkaline phosphatase, SGOT, SGPT and total protein.
The heart, liver, spleen, kidneys, adrenals, thyroid (with parathyroids), pituitary, testis, ovary and brain were removed and weighed. The following tissues/organs were fixed for subsequent histopathological examination:

Heart	Sacculus rotundus	Urinary bladder
Lungs	Colon	Adipose tissue
Bronchi	Thymus	Mammary gland
Trachea	Spleen	Brain (cerebrum,
Thyroid	Liver	cerebellum, pons)
Parathyroids	Pancreas	Pituitary
Cervical lymph nodes	Kidneys	Spinal cord
Salivary gland	Adrenals	Skeletal muscle
Tongue	Vagina	Sciatic nerve
Esophagus	Seminal vesicles	Skin (treated and untreated)
Stomach	Testes/ovaries	Bone
Duodenum	Epididymides	Bone marrow
Jejunum	Prostate/uterus	Eyes
Ileum	Mesenteric lymph nodes	Gross lesions

Statistical analyses:
Body weight, clinical chemistry and absolute and relative organ weight data were compared to control group data of the same sex using a two-tailed Student's t-test at the 5% probability level.

Result : A high dose and a low dose male died during the study and a low dose female was sacrificed in extremis. None of these deaths were considered to be treatment-related. There were no treatment-related clinical signs during the study. The animals weighed approximately 3 kg at the start of the study and mean weight gains (kg) over the course of the study are shown below:

	Mean weight gain (kg)	
Dose level	**Males**	**Females**
Control	0.4	0.2
250 mg/kg	0.2	0.5
500 mg/kg	0.1	0.1
1000 mg/kg	0.1	0.0

Although treatment seemed to have a slight effect on weight gain, the report concluded that such differences (0.1 or 0.2 kg) are quite common in rabbits of the age used in the study and that no definitive conclusions could be drawn.

Treatment caused skin irritation to varying degrees depending on dose level. A mean irritation score (mean of the sum of all irritation scores) was calculated for each group and the results are tabulated below.

Group/sex	Mean irritation Score	Classification
Control (M)	0.0	Non irritant
Control (F)	0.0	Non irritant
250 mg/kg (M)	2.3	Moderate irritant
250 mg/kg (F)	2.1	Moderate irritant
500 mg/kg (M)	3.8	Moderate irritant
500 mg/kg (F)	4.8	Moderate irritant
1000 mg/kg (M)	5.5	Severe irritant
1000 mg/kg (F)	5.3	Severe irritant

There were no treatment-related effects on either the hematological or clinical chemical determinations. Minor differences found were incidental and not dose-related. There was a reduction in left ovarian weight of the high dose females (0.129 compared to 0.193 g), but in the absence of any corresponding histopathological data was deemed to be within normal biological variation. No other treatment-related effects on absolute or relative organ weights were found.
The only treatment-related findings at gross pathology were those at the treated skin site. There were no other gross findings that were considered to be related to exposure to test material. The treated skin was described as dry, reddened, flaky, cracked, fissured and/or leathery and thickening of the dermis was also frequently noted. These changes were only seen in the exposed groups and not in the controls.
Microscopic examination found moderate to severe proliferative and moderate to severe inflammatory changes present in the skin of all animals in the high dose group (except in the animal that died early). Concurrently with these changes there was an increased granulopoiesis of the bone marrow. This was attributed to stress of the animals due to the severe skin irritation.
Two male rabbits in the high dose group had multifocal areas of hypoplasia of some of the seminiferous tubules. These testicular changes were considered to be secondary to the skin changes and not a direct effect of the test material.

Reliability : (1) valid without restriction

(14)

Species : Rabbit
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : Up to 4 weeks

Result : The following table summarizes the results of all five 28-day studies dermal studies in rabbits.

Sample API No.	Dose*	Skin Irr'n**	Growth rate/mortality	API report
Predominantly aromatics				
83-07	250	mod.	2/10 died	
	500	mod.	no effect	32-32751
	1000	sev.	1/10 died	
83-08	200	mod.	2/10 died	
	1000	sev.	weight loss	32-32753
	2000	sev.	2/10 died, weight loss	
Predominantly saturates				
83-11	200	sl.	no effect	
	1000	mod.	no effect	32-32747
	2000	mod.	weight loss in females	
81-09	200	sl.	no effect	
	1000	mod.	no effect	30-32298
	2000	mod. to sev.	no effect	
81-10	200	sl.	no effect	
	1000	mod.	no effect	30-32296
	2000	sev.	6/10 died	

* Dose given in mg/kg/day
** sl = slight
mod = moderate
sev = severe

(6) (7) (13) (14) (15)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 28 days
Frequency of treatm. : Once daily, five days each week for 4 weeks
Doses : 0.05, 0.25 & 1.0 ml/kg/day
Control group : Yes
Year : 1992
GLP : Yes
Test substance : Gas oil sample F-188 (predominantly saturate)
Sample F-188 is a gas oil containing
86% saturates
14% aromatics

Method : Three groups of ten male and ten female young adult Sprague-Dawley rats were administered test material to the shorn dorsal skin once daily, five days per week for four weeks at doses of 0.05, 0.25 or 1 ml/kg/day. The applied material was covered with an occlusive patch for six hours. A further group of ten male and ten females served as sham-treated controls. The animals were observed twice daily for clinical signs of toxicity. Dermal irritation at the application site was assessed daily prior to the next application of test material. An assessment of dermal irritation was also made 24 hours after the final application, just prior to necropsy.
Body weights were recorded three times weekly and just prior to necropsy.
At necropsy, a blood sample was taken for the following hematological and clinical chemical determinations:
Hematology

- Erythrocyte count
- Total leucocyte count
- Differential leucocyte count
- Hemoglobin
- Hematocrit
- Platelet count
- Mean corpuscular volume (MCV)

Clinical chemistry

Sodium	Glucose
Potassium	Blood urea nitrogen
Alkaline phosphatase	SGOT
SGPT	Chloride
Calcium	Phosphorus
Total protein	Creatinine
Cholesterol	Triglyceride
Albumin	Globulin (calculated)
A/G ratio (calculated)	

The following organs were weighed:

Liver
Kidneys (2)
Testes (2)/Ovaries (2)
Brain
Adrenal glands (2)

The following tissues were taken, were fixed and prepared for microscopic examination.

Accessory genital organs	Lungs with trachea
prostate	Mammary glands
seminal vesicles	Pancreas
epididymis	Peripheral nerve
Adrenal glands (2)	Pituitary
Aorta	Rectum
Brain	Salivary gland
cerebrum	Skeletal muscle (thigh)
cerebellum	Skin
pons	(treated and untreated)
Cecum	Spinal cord (cervical, mid thoracic & lumbar)
Cervical lymph nodes	Colon
Duodenum	Esophagus
Spleen	Eyes (2)

Sternum with bone marrow	Femur with articular surface
Stomach	Gross lesions and masses
Testes/ovaries (2)	Heart
Thyroid gland with	Ileum
parathyroids	Jejunum
Thymus	Kidneys (2)
Urinary bladder	Liver
Uterus	Vagina

Histopathology was done on the sham treated control group and the high dose group animals only.

Result : There were no mortalities during the study and there were no dose-related clinical observations except for the occurrence of skin irritation in all treatment groups.
Body weights were unaffected by exposure to the test material. No dermal irritation was observed in the sham-treated controls. Very slight dermal irritation was noted in both males and females in the lowest dose group (0.05 ml/kg/day). This consisted of slightly dried skin, very slight erythema and slight eschar.
In the 0.25 ml/kg/day group, irritation was slightly more and consisted of: very slight to slight (primarily very slight) erythema, slight to extreme (primarily slight to moderate) eschar and slight to moderate dried skin. Slight edema was seen in one female with slight ulceration noted in two females.
Moderate dermal irritation was seen in both males and females in the highest dose group (1 ml/kg/day). This consisted of very slight to severe erythema, slight to extreme eschar, slight to extreme dried skin, slight to extreme ulceration and very slight to slight edema.
At gross necropsy, the only treatment-related finding was skin irritation.

There were no treatment-related hematological findings.
Although differences were found in the globulin concentration and the A/G ratio they were not considered to be significant since they were not associated with any other findings and also fell within the range of normal values for Sprague-Dawley rats of the same age.
Organ weights, organ weight/body weight ratios and organ/brain weight ratios were unaffected by exposure to the test material.

The only tissues examined histologically were those from the controls and high dose group animals.
Apart from findings in the skin, there were no other treatment-related findings. The skin findings consisted of acanthosis, epidermal crusting, erosion, fibrosis, hyperkeratosis and ulceration. The incidence and severity of the lesions was greater in the high dose group than in the control group.

It was concluded that the NOAEL with respect to skin irritation was less than 0.05 ml/kg/day and the NOAEL for systemic toxicity was 1 ml/kg/day.

Reliability : (1) valid without restriction

(60)

Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : 13 Weeks
Frequency of treatm. : 5 Days each week for 13 weeks
Doses : 8, 25, 125, 500 & 1250 mg/kg

Control group	:	Yes
Year	:	1985
GLP	:	Yes
Test substance	:	Mobil Light Cycle Oil (LCO) (predominantly aromatic) See section 1.1.1.
Method	:	Light Cycle Oil (MEHSL Sample No. 8281), was applied to the clipped backs of groups of 10 male and 10 female Sprague-Dawley rats (males approx 230g and females approx 180 g at start of study). Application was 5 days/week for 13 weeks at doses of 8, 25, 125, or 500 mg/kg/day, and to an additional group of 10 males and 10 females for 2 weeks at a dose level of 1250 mg/kg/day. Males weighed approximately 240 g and females weighed approximately 180 g at the beginning of the study. The rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test article which was applied to the skin undiluted and not covered with any dressing. A similar group of 10 male and 10 female Sprague-Dawley rats served as controls. They were treated the same as the test animals, except that no material was applied to their skin. Assessment for toxic response included daily clinical observations and weekly body weight measurements. After 13 weeks the rats were killed and were carefully examined for grossly visible changes. Selected organs were weighed. Histopathological evaluation was limited to the small intestine, gonads, liver, kidneys, treated skin, spleen, stomach, thymus and urinary bladder for the control animals and those given 500 mg/kg test material per day. Blood samples were taken at 4 and 13 weeks for hematologic and serum chemical analyses. The statistical methods that were used were not specified in the report.
Result	:	Administration of test material at 500 and 1250 mg/kg/day resulted in systemic toxicity. At the high dose males and females gained less weight than controls whereas at 500 mg/kg/day only the males were affected. The animals in the high dose group looked so poorly that they were killed at the end of the second week of treatment. The females at 500 mg/kg/day and the males at 125 mg/kg/day had slightly lower body weights than the respective controls but the investigators were not clear as to whether this was a compound-related effect. LCO also caused marked, persistent effects at the site of application; severe erythema and edema with visibly thick, stiffened skin were observed. Microscopic examination of the skin from the 500 mg/kg/day group revealed moderate chronic inflammatory changes of the skin and hair follicles. The thymus was the most affected organ. In the 500 mg/kg/day group, thymus size (visually judged) and weight for both males and females was smaller than controls. Males were more affected than females. At the 125 mg/kg/day dose level only, males had slightly reduced thymus weights. The investigators judged these thymus weight differences to be attributable to a depletion of lymphocytes within the thymus. The livers of the male and female 500 mg/kg/day groups were slightly larger than controls. Also the males at this dose level had more fat in the liver cells than the controls. The investigators concluded that the effects on body and thymus weights followed a dose-response pattern. They judged the NOAEL for males and females to be 25 and 125 mg/kg/day respectively.
Reliability	:	(4) not assignable The report did not contain tables of actual data. Comments in the results section in this robust summary are taken directly from the text of the report.

The hematological and clinical chemical parameters that were measured are not specified in the report. Since raw data are not presented in the publication, it is not possible to assign a reliability to this study. However, it does provide sufficient information for a conclusion to be made on the repeat-dose toxicity of the test material.

(56)

Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	13 weeks
Frequency of treatm.	:	Once a day, 5 days each week for 13 weeks
Post exposure period	:	
Doses	:	30, 125, 500 & 2000 mg/kg
Control group	:	Yes
NOAEL	:	< 30 mg/kg
Year	:	1991
GLP	:	No data
Test substance	:	Coker light gas oil (Predominantly saturate): See section 1.1.1.

Method :

Test material was applied to the shorn skin of groups of 10 male and 10 female rats (approximately 40 days old) at dose levels of 30, 125, 500 and 2000 mg/kg. A group of 10 rats of each sex served as controls.
The test material was applied each day, 5 days each week for 13 weeks except for the 500 and 2000 mg/kg groups that were sacrificed in weeks 9 and 2 respectively. All rats were fitted with Elizabethan collars to prevent ingestion of test material. The collars were removed at the end of each week and any residual test material removed from the skin by wiping. Collars were replaced on Mondays before commencement of dosing for the next week.
Body weights were recorded before application of the first dose of test material and weekly thereafter. There were daily observations for clinical signs of toxicity and an assessment and scoring of the treated skin site was made once each week according to the standard Draize scale. Urine samples were collected during weeks 5 and 13 for urinalysis (pH, specific gravity, bilirubin, urobilinogen, blood, protein, glucose and ketone). Blood samples were taken at the end of the study for the determination of the following clinical chemical and hematological parameters.

<u>Hematology</u>

Red cell count	Hemoglobin
Hematocrit	White cell count
Platelet count	

<u>Clinical chemistry</u>

Sorbitol dehydrogenase	Cholesterol
Alanine aminotransferase	Urea nitrogen
Aspartate aminotransferase	Total protein
Alkaline phosphatase	albumin (A)
Bilirubin	Triglycerides
Inorganic phosphorus	Creatinine
Glucose	Uric acid
Sodium	Potassium
Chloride	Calcium

Globulin(G) and A/G ratios were calculated

All animals surviving to the end of the study were sacrificed and necropsied. The following organs were weighed:

Adrenals	Heart	Spleen
Brain	Kidneys	Thymus
Liver	Ovaries	Uterus
Prostate	Epididymides	Testes

The following tissues/organs were removed from control group and high dose group animals and were fixed for subsequent histopathological examination.

Adrenals (both)	Ovaries (both)
Bone and marrow (sternum)	Pancreas (head)
Brain (3 sections)	Salivary gland (submaxillary)
Eye (left & optic nerve)	Skin (treated 2 sections)
Heart	Spleen
Colon	Stomach (squamous & glandular)
Duodenum	Thymus (both lobes)
Kidneys (both)	Thyroid (both lobes)
Liver (2 lobes)	Urinary bladder
Lung (left lobe)	Uterus (body & horns)
Skeletal muscle (thigh)	Gross lesions
Peripheral nerve (sciatic)	

In addition the following tissues/organs were removed, fixed and examined microscopically from the mid and low dose animals:

Adrenals	Sternum (bone and marrow)
Kidneys (both)	Liver (2 lobes)
Lung	Skin (2 sections plus any gross lesions)
Thymus	Gross lesions.

At the end of the study the epididymides and testes from the male rats in the control and 125 mg/kg groups were removed. Prior to sample preparation for testis examination, the tunica albuginea and corresponding blood vessels were removed and discarded before the remaining testicular parenchyma and cauda epididymis were weighed. Testes were prepared for spermatid count and epididymides were prepared for spermatozoa count and a morphological assessment was made of testes and epididymides.

Statistical analysis
Body weight, serum chemistry, hematology and organ weight data were analyzed by parametric methods: analysis of variance and associated F-test, followed by Tukey's multiple comparison test (body weight, hematology and organ weight data) or Student-Newman-Keuls multiple comparison test (serum chemistry), provided that there was statistical significance in the analysis of variance.
Differences between control and treated groups were considered statistically significant only if the probability of the differences being due to chance was less than 5% ($P<0.05$).

Result : The animals in the 2000 and 500 mg/kg groups were sacrificed during weeks 2 and 9 respectively due to severe skin irritation and moribund condition.
Skin irritation, generally severe, was seen in all treated animals. Apart from the observation of perineal staining, which was seen in all groups,

there were no remarkable clinical findings during the study.
During the weeks they were on the study, body weights of the 2000 and 500 mg/kg groups for both sexes was significantly less than controls. Body weights of males in the 125 mg/kg group were less than controls from day 36 onwards and although there were three occasions on which the male body weights in the 125 mg/kg group were also reduced there were no other effects on growth rates.
Results of urinalysis were in general unaffected by exposure to the test material.
Statistically significant differences between treated and control clinical chemical analyses at 13 weeks are summarized below. Only the results at 13 weeks are shown, the 5 week results are NOT included.

Parameter % change compared to control

	30 mg/kg		125 mg/kg	
	Male	Female	Male	Female
Uric acid	-	-	-	-
Glucose	-12%	-	-17%	-20%
Urea Nitrogen	-	-	-	+21.6%
AST	-33%	-	-31%	-
ALT	-	-	-	-
Alk. Phos.	-	+30%	-	+35%
Creatinine	-	-	-	-
Cholesterol	-	-	-	-
Triglycerides	-	-	-	-
Total protein	-	-	-	-
Bilirubin	-	-	-	-
Albumin	-	-	-	-
Calcium	-	-5%	-	-6%
Phosphorus	-	-	-	-
Sodium	-	-	-	-
Potassium	-	-	-	-
Chloride	-	-	-2%	-
A/G ratio	-	-	-	-
Globulin	-	-	-	-
SDH	-	+40%	-	-30%

At 13 weeks the hematological parameters affected were:

* an increase in WBCs and the number of segmented neutrophils in the high dose males and females
* an increase in lymphocytes in the 125 mg/kg group of both sexes and the 30 mg/kg group females.

No effects were found in any of the sperm evaluations that were made.
The only effect noted in the absolute and relative organ weight data of the 30 mg/kg group was a reduction of approximately 10% in the absolute thymus weight in males.

In the 125 mg/kg group there were more differences noted in organ weights and these are tabulated below as percentage change. The authors judged that the decreases in organ weights were probably due to the reduced body weights of the animals. However, they also judged that the effect was probably compound related for those organs for which there were differences in organ/body weight ratio.

A = Absolute weight, R= Organ/body weight ratio

Organ		Male	Female
Adrenals	(A)	-	-
	(R)	+29%	-
Brain	(A)	-	-
	(R)	+11%	-
Epididymides	(A)	-	-
	(R)	+11%	-
Heart	(A)	-	-
	(R)	-	+6%
Kidneys	(A)	-	-
	(R)	+8%	+8%
Liver	(A)	-	-
	(R)	+13%	+22%
Prostate	(A)	-	
	(R)	+17%	
Spleen	(A)	-	-
	(R)	+19%	+19%
Testes	(A)	-	
	(R)	+11%	
Thymus	(A)	-34%	-23%
	(R)	-25%	-
Uterus	(A)		-
	(R)		-
Ovaries	(A)		-
	(R)		-

The primary treatment-related changes observed at histopathological examination were severe skin irritation and slight effects on bone marrow and kidneys.

The bone marrow effects included:

* at 2000 mg/kg a severe reduction in erythropoietic cells and megakaryocytes
* at 2000, 500 and 125 mg/kg megakaryocyte changes characterized by larger, vacuolated, and/or darkened nuclei or clumped cell effects

The kidney effects included:

* Basophilia in the tubular cortex, predominantly in males. Focal inflammation, dilation of ducts in the medulla and tubules in the cortex.

Changes were also seen in the adrenals, liver, lungs, draining lymph nodes, prostate, seminal vesicles, spleen, thymus and uterus. However, these changes were considered to be secondary effects probably due to reduced weight gain, treatment-related skin injury, slight septicemia and stress.

Reliability : (1) valid without restriction

(59)

Type : Sub-chronic
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 6 hours
Frequency of treatm. : Daily, five days each week for four consecutive weeks

Doses : Nominal 25 mg/m³
Control group : Yes
Year : 1986
GLP : Yes
Test substance : API 81-09 & API 81-10 [predominantly saturates]

Method : Groups of 20 male and 20 female Sprague Dawley rats (aged approximately six weeks) were exposed to a nominal concentrations of 25mg/m³ of each of two samples of hydrodesulfurized middle distillate by inhalation. Exposures were for approximately six hours each day, five days each week for four consecutive weeks. Control groups of 20 male and 20 females were exposed to filtered air.
Animals were observed twice daily for overt signs of toxicity and they underwent detailed examination once weekly. Body weights were also recorded weekly.
At study termination, the animals were killed and blood samples were taken for the following clinical chemical and hematological investigations:

Hematology	Clinical chemistry
Hematocrit	Aspartate aminotransferase
Hemoglobin	Alanine aminotransferase
Erythrocyte count	Alkaline phosphatase
MCH	Glucose
MCV	Urea nitrogen
MCHC	Total protein
Leucocyte count	
Platelet count	
Reticulocyte count	

For all rats, the following organs were weighed and the organ body weight ratios were calculated :
Heart, lung and trachea, liver, kidneys, brain, spleen, adrenals, thyroid/parathyroid, pituitary, testes and ovaries.
The following tissues were removed and preserved:

Adrenals (2)	Aorta
Bone marrow (femur)	Bone marrow smear
Brain (3 levels)	Eye with contiguous Harderian gland
Esophagus	Stomach
Duodenum	Jejunum
Ileum	Cecum
Colon	Rectum
Gonads	Ovary (2)
Kidney (2)	Testis with epididymis (2)
Heart	Liver (3 sections)
Nasal tissues	Lung & trachea (all lobes)
Abdominal lymph nodes	Thoracic lymph nodes
Mammary region lymph nodes	
Pancreas	Pituitary
Sciatic nerve	Prostate & seminal vesicle
Skeletal muscle (thigh)	Skin
Salivary gland (mandibular with submandibular lymph node)	
Spinal cord (cervical, mid thoracic & lumbar)	
Spleen	Thymic region
Thyroid/parathyroid complex	
Urinary bladder	Uterus (2 horns & cervix)
Vagina	

On all rats the following tissues were examined microscopically:
Adrenal (2), brain (3 levels: fore, mid & hind), bronchi, esophagus, eye (2), heart, kidney (2), liver, lungs (2), lymph ode (mediastinal), ovary (2), pancreas, pituitary, prostate, salivary gland, skin, spleen, stomach, testis (2), thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, all gross lesions.

Statistical analysis
Body weight, hematology, clinical chemistry and organ weight data were analyzed by analysis of variance and Bartlett's test.
Treatment groups were compared to control by sex, using the appropriate t-statistic.
Data containing inequalities or where group variances were heterogeneous were compared using a non-parametric approach, by transforming the data into ranks prior to analysis as described by Conover and Iman.

Result : There were no treatment-related clinical observations during exposure, nor were there any effects on body weight.
Although there were some minor clinical chemical differences, these were considered to be unrelated to treatment.
The only noteworthy effect was an increase in leukocyte counts in males and females (29 % & 31 % respectively) exposed to sample API 81-10.
There were no macroscopic observations at necropsy.

Although there were some organ weight/relative organ weight differences, they were not considered relevant in the absence of any related microscopic pathology.

Microscopic tissue changes were confined to the nasal tissues of males and females exposed to API 81-09.
The changes consisted of subacute inflammation (Rhinitis) of the respiratory mucosa. The incidence and severity are shown in the following table.

	Male		Female	
	Control	**81-09**	**Control**	**81-09**
No. examined	20	20	20	20
Inflammation				
trace	0	7	0	3
mild	0	10	0	12

No other treatment-related changes were observed in any treatment group.

Test condition : Atmospheres were generated by atomizing the test material into an atomization chamber. The resulting vapors/aerosols were directed to the chamber inlet where dilution with chamber ventilation air reduced the concentration to the desired level.
Nominal concentrations were calculated from test material use rates.
Actual concentrations were determined by standard gravimetric techniques.
Aerosol particle size was also determined.
The nominal and actual concentrations for the study were:

		Exposure concentration (mg/m³)			
Sample	**Desired conc. mg/m³**	**Nominal**		**Actual**	
		Mean	**SD**	**Mean**	**SD**
API 81-09	25	127	16.8	23	4.41
API 81-10	25	160	68.1	24	9.73

Particle size determinations showed the following:

	Week	EAD* (mcm)	GSD**
API 81-09	1	3.6	2.03
	3	3.5	2.19
API 81-10	1	3.2	2.10
	3	3.3	2.11

* EAD = Equivalent Aerodynamic Diameter
** GSD = Geometric standard deviation

Reliability : (1) valid without restriction

(39)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Modified Ames bioassay
System of testing : S. Typhimurium Strain TA98
Test concentration : 1 to 60 µl
Metabolic activation : With and without
Year : 1991
GLP : No data
Test substance : 14 DGMK samples of gas oil (DMK 1-14 inc) See section 1.1.1.

Method : A modified Salmonella mutagenicity assay was performed at the Mobil Environmental and Health Science Laboratory. The technique that was used has been described fully elsewhere (Blackburn et al 1984 & 1986).

The middle distillate samples (2 ml) were dissolved in cyclohexane and the solution was then extracted with DMSO (10 ml). These extracts were tested in Salmonella typhimurium strain TA98.
The concentrations of DMSO extract used were: 60, 50, 40, 30, 20 15, 10 and 5 µl/60 µl. Extra concentrations were used for some assays.
Positive controls were 2.0 µg 2-aminoanthracene, 10.0 µg benzo(a)pyrene and 25 µg 2-nitrofluorene in 50 µl DMSO per bacterial plate.
Metabolic activation was accomplished by using an eight-fold higher concentration of the liver S9 fraction obtained from Arachlor-induced Syrian Hamsters rather than rats.
NADP cofactor was also increased from the normal 4 to 8 mM.

Result : A mutagenicity index (MI) was calculated, which represented the slope of the dose response curve for each of the samples.
Previous studies have established that materials with an MI of less than or equal to 1.0 have not been associated with a tumorigenic response in skin painting bioassays, whereas those materials with an MI greater than 1.0 have been associated with a tumorigenic response.
The MIs for the 14 middle distillate samples were:

Sample	PAC content*	Mutagenicity Index	Aromatic content
Predominantly aromatic			
10	8.3	9.3	59.8
7	11.3	9	55.1
14	5.4	7.6	52.4
Predominantly saturates			
6**	4.2	4	48.3
13**	2.4	2.3	46.9

12**	3.7	3.1	42.6
9	4	4	42.5
5	4.7	1.4	33.9
2**	4	1.3	32.6
4	1.4	0.7	29.3
3	2.8	1	28.1
8	4	2.1	27
1	1.8	0.8	23.6
11**	1	0.7	21

* Total weight % PAC
** Sample contains olefins

Test substance : A total of 24 samples were tested in this study as follows.

Straight run gas oils	Samples 1 - 5 inc.
Cracked gas oils	Samples 6 - 14 inc.
Light fuel oil	Samples 15 - 21 inc
Diesel fuel	Samples 22 - 24

This summary only includes the results on the gas oils (samples 1-14 inc). Results of the studies on the fuels (samples 15-24 inc) are summarized in the robust summaries on distillate fuels.

Reliability : (1) valid without restriction

(41) (42) (46)

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Test concentration : 5 to 80 nl/ml without activation and 2.5 to 30 nl/ml with activation
Metabolic activation : With and without
Method : OECD Guide-line 476
Year : 1985
GLP : Yes
Test substance : API 83-07 (See section 1.1.1.)

Method : The test material was dissolved in ethanol for this assay.
Two positive control substances were used viz ethyl methane sulphonate (EMS) at concentrations of 0.25 & 0.4 µl/ml and 3-methylcholanthrene (MCA) at concentrations of 2.5 & 4.0 µg/ml.

A cytotoxicity study carried out prior to the mutagenicity assay established that the sample was highly toxic at 62.5 nl/ml without activation and lethal at the same concentration in the presence of metabolic activation.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at dose levels up to 120 nl/ml without activation and up to 60 nl/ml with S-9 activation.
After exposure to the test material, the cells were allowed to recover for 2 days and then cultures were selected for cloning and mutant selection. Plates containing colonies of selected cells were incubated for 10 to 14 days after which they were scored for total number of colonies per plate. A mutation frequency was then determined.

Result : After the 2 day recovery period, seven non-activated cultures and seven S-9 activated cultures were cloned based on their degree of toxicity. The mutant frequencies and the percentage total growth at each of the test concentrations is summarized in the following table.

Concentration (nl/ml)	Mutant frequency	Relative growth %
Non-Activated		
5	34	34.2
15	19.1	67.5
30	32.2	81.1
40	21.9	153.8
50	18.9	55.5
60	41.8	33.8
80	Excessive toxicity, treatment not cloned	
Solvent 1	33.5	100
Solvent 2	19.2	100
Solvent 3	30.1	100
EMS 0.25 µl/ml	426.1	38.2
EMS 0.4 µl/ml	570.2	58.3
S-9 Activated		
2.5	52.5	66.2
5	67	74.8
10	158.5	64.8
15	198.5	22.3
20	207.6	12.8
25	255.3	6.7
30	Excessive toxicity, treatment not cloned	
Solvent 1	48	100
Solvent 2	56.5	100
Solvent 3	55.7	100
MCA 2.5 µg/ml	209.3	78
MCA 4 µg/ml	411.2	33.1

According to the criteria used by the authors to judge the activity of the test material, the sample produced a positive response in the presence of S-9 activation but was not mutagenic in the absence of activation.

Reliability : (1) valid without restriction

(23)

Type : Mouse lymphoma assay
Test substance : other TS: various gas oils (see section 1.1.1.)

Result : Mouse Lymphoma assays have been carried out on 6 different PI samples, including API 83-07. Te results for all API samples are summarized below.

API sample	Result
Predominantly aromatic samples	
83-07	Positive with S9 activation Negative without S9 activation (Ref API 32-32167)
83-08	Positive with and without S9 activation (Ref API 32-31709)
81-10 (Aromatic fraction)	Negative with and without S9 activation (Ref API 34-32644)

Predominantly saturates

83-11	Laboratory 1 (Ref API 32-32166) Positive with S9 activation Negative without S9 activation
	Laboratory 2 (Ref API 32-31768) Positive with and without S9 activation
81-09	Positive only at high toxicity without S9 activation (Ref API 32-30965)
81-10	Test 1 (Ref API 32-30535) Positive at moderate to high toxicity with S9 activation only
	Test 2 (Ref API 33-31224) Weakly positive with and without S9 activation
	Test 3 (Ref 34-32643) Positive with S9 activation only
	Saturates fraction (Ref API 34-32645) Negative with and without S9 activation

(12) (20) (21) (22) (23) (24) (28) (31) (32) (33)

Type : Sister chromatid exchange assay
System of testing : Chinese Hamster Ovary Cells (CHO)
Test concentration : 0.1 to 1000 µg/ml
Metabolic activation : With and without
Result : Ambiguous
Year : 1988
GLP : Yes
Test substance : API 83-07 (See section 1.1.1.)

Method : A cytotoxicity study was performed in order to select dose levels for the SCE assay.
For the SCE assay CHO cells were seeded in duplicate for each treatment condition and were incubated at 37°C in a humidified atmosphere for 16 to 24 hours. Treatment was carried out by refeeding two complete sets of flasks with complete medium for the non activation study or with S-9 reaction mixture for the activated study to which was added 50 µl of dosing solution of test control or article in solvent or solvent alone. In the non-activation study the cells were exposed for about 26 hours. Two hours after exposure 0.01 mM BrdUrd was added to the treatment medium. At the end of the treatment period, the treatment medium was removed, the cells rinsed and then exposed to colcemid (0.1 µg/ml) for a further 2 hours. In the activation study exposure was for 2 hours. After the exposure period, the treatment medium was removed, the cells were washed re-fed with medium containing BrdUrd and then incubated for a further 26 hours. Colcemid was added for the last 2 hours of incubation.

For activated and non-activated assays metaphase cells were harvested 2 hours after addition of colcemid. Cells were collected and fixed and stored until slides were prepared.

Slides were coded and scored without regard to treatment group. Only cells with 20 ± 2 centromeres were selected for evaluation of SCEs. A total

of 4 doses were scored including the highest test article dose where sufficient second-division metaphase cells wee available. SCEs were scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. The percentage of cells in first (M1), second (M2) or third division (M3) metaphase was also recorded for a total of 100 metaphase cells scored. TEM was used as positive control at a concentration of 0.025 µg/ml. in the non-activated assay. In the activated assay CP was used as a positive control at a concentration of 2.5 µg/ml.

A test was deemed valid if the mean SCE/cell in the untreated control did not exceed 13 and the mean SCE/cell for the positive control was at least double that of the negative control.

A test material is considered positive if it induces a doubling in SCE frequency over the solvent control at a minimum of three consecutive dose levels or if a dose responsive and statistically significant increase is observed over a minimum of 3 dose levels.

Remark : In a separate Sister Chromatid Exchange assay, Sample API 81-10 (sample containing predominantly saturates) was negative without S9 activation. When tested with S9 activation, the result was equivocal and there was no dose relationship. (Ref API 35-32433)

Result : In the non-activation assay the two highest dose levels could not be evaluated due to severe cell delay and the absence of scorable second-division metaphase cells. Since only two dose levels were scorable and they were both significantly greater than the solvent controls. A repeat study was conducted but at dose levels of 2.5, 5, 10, 20 and 30 µg/ml. However, only the four highest doses were scored for SCEs. The results of both studies are summarized below.
Statistically significant differences are indicated:
* P less than or equal to 0.05 by Student's t test
** P less than or equal to 0.01 by Student's t test

Treatment/Replicate		SCEs/ chromosome	Group mean SCEs/cell
Non-activation assay (FIRST study)			
Untreated cells	A	0.51	
	B	0.61	11.00 ±3.9
Acetone	A	0.62	
	B	0.58	11.82 ±2.76
API 83-07			
10 µg/ml	A	0.61	
	B	0.78	13.68 ±4.43**
20 µg/ml	A	0.65	
	B	0.7	13.26 ±3.95*
40 µg/ml	A	Not determined	
	B	Not reported	
80 µg/ml	A	Not determined	
	B	Not determined	
TEM	A	3.06	
	B	3.49	63.86±13.07**

Non-activation assay (REPEAT study)

Untreated cells	A	0.55	
	B	0.55	10.74 +/-3.02
Acetone	A	0.57	
	B	0.59	11.42 +/-3.49
API 83-07			
5 µg/ml	A	0.60	
	B	0.49	10.70 +/-3.33
10 µg/ml	A	0.57	
	B	0.60	11.52 +/-3.56
20 µg/ml	A	0.63	
	B	0.57	11.76 +/-2.79
30 µg/ml	A	0.75	
	B	0.63	13.40 +/-4.84*
TEM	A	1.60	
	B	1.57	31.00 +/-6.38**

In the repeat study the frequency of SCEs was significantly greater than the solvent controls in the high dose only.

The results from the activation assay were as follows:

Activation assay

Untreated cells	A	0.62	
	B	0.62	12.20 +/-3.99
Solvent	A	0.67	
	B	0.67	13.16 +/-3.88
API 83-07			
10 µg/ml	A	0.76	
	B	0.76	14.86 +/-4.60*
20 µg/ml	A	0.65	
	B	0.71	13.44 +/-3.36
40 µg/ml	A	0.67	
	B	0.80	14.60 +/-4.13*
40 µg/ml	A	0.82	
	B	0.77	15.58 +/-4.34**
80 µg/ml	A	0.82	
	B	0.77	15.58 +/-6.34**
Cyclophosphamide	A	1.57	
	B	1.63	31.56 +/-6.34**

In this assay the increases in frequency of SCEs at all but one dose level were significantly greater than that for the solvent controls.

Conclusion : The authors concluded: The positive and negative controls fulfilled the requirements for a valid test.

Under the conditions described in the report, API 83-07 did induce an increase in SCEs in CHO cells at one or two concentrations in two independent studies when tested in the absence of exogenous activation and at three non-consecutive concentrations when tested in the S-9 activated system in a single study. In the non-activated test system, activity at a single concentration was not reproducible. Because the increase in SCEs above the spontaneous background level had no clear dose-response, API 83-07 was concluded to be equivocal in this test

system.

Reliability : (2) valid with restrictions

(35) (36)

Remark : In a separate Sister Chromatid Exchange assay, Sample API 81-10 (sample containing predominantly saturates) was negative without S9 activation. When tested with S9 activation, the result was equivocal and there was no dose relationship. (Ref API 35-32433)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : 6, 24 and 48 hours
Doses : 2, 0.67 & 0.2 g/kg
Result : Negative
Method : OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year : 1986
GLP : Yes
Test substance : API 83-07 (Sample consisting predominantly of aromatics (See section 1.1.1.)

Method : Undiluted test material was given intraperitoneally to groups of 15 rats of each sex at three different dose levels (0.2, 0.67 & 2.0 g/kg). A group of 15 rats of each sex, serving as negative controls, were given deionized water. A group of 5 animals of each sex, used as positive controls was dosed with 1.0 mg/kg triethylenemelamine (TEM) and these animals were killed 24 hours afterwards. Three hours prior to being killed the rats were given a single i.p dose of colchicine (4 mg/kg).
For each dose level of test material and the negative controls 5 rats of each sex were killed 6, 24 and 48 hours after dosing.
Immediately following sacrifice bone marrow was aspirated from the tibiae. The marrow was washed and the cells were fixed before being spread on slides for examination (routinely 50 spreads for each animal). Slides were stained and scored for chromatid and chromosome gaps and breaks, fragments, structural rearrangements and ploidy. A mitotic index based on at least 500 cells was recorded. The index was calculated by scoring the number of cells in mitosis per 500 cells on each slide read.

The data on chromosomal aberrations for the treated animals was compared to that for the negative controls.
The criteria used in assessing the result are described in the results section.

Result : Immediately after dosing all animals in the 2 g/kg group were lethargic. There were some mortalities in the high dose group and where possible they were replaced. Throughout the study lethargy was observed in the high dose group animals but no toxic signs were seen in any of the other dose groups.
A summary of the chromosomal results is given in the following table.

Group	Time	Frequency of aberrations	% cells 1+ aberrations Str*	% cells 2+ aberrations Num**	Mitotic index
Male					
-ve control					
6	0	.024	0	0	4.1
24	.004	.016	.4	0	2.8
48	.01	.02	1	0	3.5
+ve control (TEM @1.0 mg/kg)					
24	>1.17	.1	29	18.8	1.3
Test material					
0.2 6	.015	.005	1.5	0	2.8
24	0	.016	0	0	4.3
48	0	.02	0	0	3.6
0.67 6	0	.04	0	0	5.9
24	>.02	.03	2	.4	4.1
48	0	.01	0	0	4.5
2.0 6	0	.028	0	0	3.8
24	.01	.01	1	0	4.7
48	0	.02	0	0	3.6
Female					
-ve control					
6	.01	.01	1	0	4.2
24	.012	.024	1.2	0	2.6
48	0	.036	0	0	4.2
+ve control (TEM @1.0 mg/kg)					
24	>4.199	0	59.9	54.5	.2
Test material					
0.2 6	0	.012	0	0	3.1
24	.004	.o4	.4	0	3.3
48	.004	.012	.4	0	2.9
0.67 6	>.016	.044	1.6	.4	4.6
24	0	.008	0	0	3.6
48	0	.016	0	0	5.5
2.0 6	0	.032	0	0	4.1
24	0	.016	0	0	4.9
48	0	.01	0	0	6.2

For simplicity only standard error values have not been shown in the above table.

The authors make the following interpretation of the results
The test material did not induce a significant increase in the percentage of aberrant cells above the controls for either sex at any of the doses or kill times. No apparent test article effects on the mitotic index were noted in any of the dose groups. The positive control (TEM) induced significant increases in the percentage of cells with structural chromosomal aberrations in the male animals (29%). The positive control also produced

a very high percentage of cells with structural chromosomal aberrations in the female dose group (59.9%). However, the data from the female positive control group could not be statistically analyzed since only 32 metaphase cells could be analyzed. The lack of analyzable metaphase cells can be attributed to bone marrow toxicity induced by the TEM.

Reliability : (1) valid without restriction

(29)

Type : Cytogenetic assay
Test substance : Gas oils various (See section 1.1.1.)

Result : A total of six bone marrow cytogenetics assays have been carried out on API gas oil samples. All of these studies have been negative.

The samples tested and the references to the original reports are:

API sample	**Reference**
Samples predominantly aromatic	
83-07	33-30929
83-08	33-30493
Samples predominantly saturates	
83-11	32-32408
	33-30930
81-09	32-30965
81-10	32-30535

(12) (16) (17) (22) (27)

Type : Sister chromatid exchange assay
Species : Mouse
Route of admin. : i.p.
Exposure period :
Doses : 340, 1700 and 3400 mg/kg
Result : Positive
Year : 1988
Test substance : API 83-07

(37)

Type : Sister chromatid exchange assay
Species : Mouse
Route of admin. : i.p.
Doses : 0.5, 2.5 and 5.0 mg/kg
Result : Negative
Year : 1988
Test substance : API 81-10

(34)

5.7 CARCINOGENICITY

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 104 weeks

Frequency of treatm. : 2, 4 or 7 days/week
Doses : Doses variable, see method
Control group : Yes, concurrent vehicle
Year : 1996
GLP : Yes
Test substance : MD-7 (See section 1.1.1.)

Method : The testing of the cracked gas oil (MD-7) was part of an overall larger study. For the purpose of this summary, only the details relating to MD-7 are presented. The test material was applied to the shorn skin of three groups of 50 male mice for 104 weeks. The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. The concentrations and frequencies were selected to determine the influence of skin irritation on the tumorigenic response.

The following dosing regimes were used

Group No.	Concentration of gas oil* (%)	µl/dose	Dosing frequency
9	100	50	2 times/week
10	50	50	4 times/week
11	28.5	50	7 times/week

* Mineral oil was used as diluent

A control group of 50 male mice received 35 µl mineral oil 7 days each week.

All animals were observed regularly for viability, clinical signs and a score was given for any dermal irritation that occurred. Body weights were recorded throughout the study. When they developed, dermal growths were measured and documented.

All animals were necropsied either when they died during the study or at the end of the study. The necropsy included an examination of the body, all orifices and the carcass, cranial, thoracic and abdominal cavities, including their contents.

For all animals, tissues were preserved and examined microscopically from all skin tumors, skin from treated and untreated sites and any grossly observable masses.

Result : Survival was less in the MD-7 treated groups compared to the negative controls; at the lower two concentrations (28.5 and 50 %) the difference was statistically significant. Dermal irritation occurred in the groups exposed to gas oil. The dermal irritation scores were:

Group	Range of scores	Mean dermal score
Negative control (oil)	0-0.22	0.06
100% gas oil 2X/week	0-4.0	2.4
50% gas oil 4X/week	0-4.0	1.59
28.5% gas oil 7X/week	0-1.67	0.28
Positive control (HCO)	0-2.0	0.73

There were no other treatment-related clinical findings.

Treatment-related findings at post mortem were limited to

Treatment did not have any adverse effect on body weights.

dermal irritation and were consistent with the findings of the in-life phase of the study.

Liver masses and gastrointestinal abnormalities were observed but these were found in all groups and were considered to be incidental.

Tumors developed in the positive control group (HCO) and in the MD-7 treated groups as follows:

Material	Oil	HCO	MD-7	MD-7	MD-7
Concentration			28.5%	50%	100%
No. applications/week			7	4	2
No. mice examined	50	50	50	50	50
No. mice with tumors	0	47	1	17	7
Tumor types					
Squamous cell carcinoma	0	42/73*	1	8/9*	3
Fibrosarcoma	0	0	1	4	0
Melanoma	0	0	0	1	0
Papilloma	0	37/88*	0	10/134*	

* / = No with neoplasms/actual incidence of neoplasms

Reliability : (1) valid without restriction

(49)

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 104 weeks
Frequency of treatm. : 2, 4 or 7 days/week
Doses : Variable, see methods
Control group : Yes, concurrent vehicle
Year : 1996
GLP : Yes
Test substance : MD-6 (See section 1.1.1.)
Sample MD-6 was a straight run, hydrotreated gas oil, with CAS No. 64742-46-7.
The sample contained 1.3% m/m 3-7 ring PAC
The vehicle control that was used was a Mineral oil Solvent 100 neutral low pour. CAS No. 64742-54-7

Method : The testing of the straight run gas oil was part of an overall larger study. For the purpose of this summary, only the details relating to the straight run gas oil are presented.
The test material was applied to the shorn skin of three groups of 50 male mice for 104 weeks.
The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. The concentrations and frequencies were selected to determine the influence of skin irritation on the tumorigenic response.

The following dosing regimes were used

Group No.	Concentration of gas oil* (%)	µl/dose	Dosing frequency
6	100	50	2 times/week
7	50	50	4 times/week
8	28.5	50	7 times/week

* Mineral oil was used as diluent
A control group of 50 male mice received 35 µl mineral oil 7 days each week.
All animals were observed regularly for viability, clinical signs and a score was given for any dermal irritation that occurred. Body weights were recorded throughout the study. When they developed, dermal growths were measured and documented. All animals were necropsied either when they died during the study or at the end of the study. the necropsy included an examination of the body, all orifices, the carcass and cranial, thoracic and abdominal cavities, including their contents. For all animals, tissues were preserved and examined microscopically from all skin tumors, skin from treated and untreated sites and any grossly observable masses.

Result : There was no significant difference between the survival of the negative controls and any of the groups receiving gas oil. Survival of the positive control group was poorer than the negative controls. Dermal irritation occurred in the groups exposed to gas oil. The dermal irritation scores were:

Group	Range of scores	Mean dermal score
Negative control	0-0.22	0.06
100% gas oil 2X/week	0-4.0	2.0
50% gas oil 4X/week	0-0.5	0.09
28.5% gas oil 7X/week	0-0.47	0.02
Positive control	0-2.0	0.73

There were no other treatment-related clinical findings. Treatment did not have any adverse effect on body weights. Treatment related findings at post mortem were limited to dermal irritation and were consistent with the findings of the in-life phase of the study.
Liver masses and gastrointestinal abnormalities were observed but these were found in all groups and were considered to be incidental.
Tumors developed in the positive control group and in two of the gas oil groups thus:

Group	Animals with skin masses	Animals with confirmed tumors
Negative control	0	0
Positive control	47	47
100% MD-6	2	4
50% MD-6	0	0
28.5% MD-6	1	1

In the group receiving undiluted MD-6 one animal had developed a basal cell carcinoma and one animal a squamous cell carcinoma. 3 mice had developed papillomas. A squamous cell carcinoma was observed in one animal receiving 28.5% MD-6 seven days a week. The tumor incidence was highest in the group in which skin irritation was greatest with no tumors developing in the middle dose group. The report suggests that the single tumor that developed in one mouse in the lowest dose group could have been spontaneous.

Reliability : (1) valid without restriction

(49)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	Days 0 to 19 of gestation
Frequency of treatm.	:	Daily
Duration of test	:	Up to gestation day 20
Doses	:	25, 50, 125, 250, 500 & 1000 mg/kg
Control group	:	Yes
Year	:	1987
GLP	:	No data
Test substance	:	Mobil Light Cycle Oil (LCO) (See section 1.1.1.)

Method :

Prior to dosing, females (approximately 12 weeks old) were paired and the appearance of a vaginal plug or the presence of spermatozoa in vaginal lavage fluid was taken to indicate that mating had occurred. This was taken to be day 0 of the study. The presumed-pregnant rats were distributed into the following groups each of 10 animals.
Light cycle oil (LCO) was applied daily to the shorn dorsal skin at the dose levels shown below and for the duration indicated. The rats were fitted with collars to prevent oral ingestion of the applied material. Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gas oil that had been applied to other animals.
The study design is outlined as follows:

	Dose level (mg/kg/day)	Dosing days*
Group 1	0 (Remote sham control)	0-19
Group 2	0 (Proximal sham control)	0-19
Group 3	25	0-19
Group 4	50	0-19
Group 5	125	0-19
Group 6	250	0-19
Group 7	500	0-19
Group 8A	1000	0-6
Group 8B	1000	6-15

* denotes days of gestation

Observations were made daily for clinical signs. Body weights and food consumption were recorded regularly throughout the study.

Each female was sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly. The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined. The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and the following clinical chemical measurements were made:
Alanine aminotransferase Glucose

Albumin	Lactate dehydrogenase
Albumin/globulin ratio	Inorganic phosphorus
Alkaline phosphatase	Potassium
Aspartate aminotransferase	Sodium
Bilirubin (total)	Sorbitol dehydrogenase
Calcium	Total protein
Chloride	Triglycerides
Cholesterol	Urea nitrogen
Creatinine	Uric acid
Globulin	

Fetuses were examined and half were preserved for examination of soft tissue abnormalities, the remainder being differentially stained for skeletal examination.

Statistical analysis
Maternal biphase data, cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's exact or Dunnet's test.
Serum chemistry data were analyzed for analysis of variance followed by comparisons using Tukey's test.

For all statistical analyses, differences between control and treated groups were considered to be significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$)

Result **:** Erythema and flaking of the skin were observed in all groups exposed to LCO. Eschar, fissuring, scabbing and scar formation was observed in all but the 25 mg/kg group. Sensory Irritation was particularly severe in the 500 and 1000 mg/kg groups.
At doses higher than 25 mg/kg there was a decrease in body weight and body weight gain compared to the controls and this was accompanied by a reduction in food consumption.

There were no treatment-related findings at necropsy.
In the clinical chemical measurements there were no differences recorded for the 1000 mg/kg animals. However, cholesterol and triglycerides were increased in the 250, 500 and 1000 mg/kg groups. The dose-response was linear only for triglycerides.
Fetal body weights were reduced only in the 500 and 1000 mg/kg groups and statistical significance was achieved only in the latter group.
The number of malformations (soft tissue and skeletal) that occurred are tabulated below

Group (mg/kg)	**No. affected/ No. examined**	**No. fetuses with >1 anomaly**	**No. litters adversely affected**
0	4/135	2	2
0	137	0	0
25	1/143	1	1
50	0/111	0	0
125	0/148	0	0
250	1/136	1	1
500	2/112	2	2

No anomalies were seen in either of the 1000 mg/kg groups.

Reliability **:** (2) valid with restrictions
The report reviewed was incomplete and only a textual description (no data tables) was given of the anomalies observed.

(58)

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Frequency of treatm.	:	Once daily
Duration of test	:	From days 0-20 of gestation
Doses	:	50, 150 & 500 mg/kg/day
Control group	:	Yes
NOAEL maternal tox.	:	< 50 mg/kg bw
NOAEL teratogen.	:	50 mg/kg bw
Year	:	1994
GLP	:	Yes
Test substance	:	Test material F-215 is a straight run gas oil and has the following composition: Saturates 65.4% Aromatics 34.6%

Method :

Prior to the conduct of the developmental toxicity screen a screening study for skin irritation was carried out.
The irritation screen was conducted in four female rats at dose levels of 250, 500 and 1000 mg/kg/day. The test material was applied undiluted daily for seven days.
No mortality was observed during this screening study and slight to extreme erythema, edema, eschar and dry skin were noted in all dose groups. Animals in the 250 mg/kg/day group gained body weight during the study.
Weights decreased for one of four animals in the 500 mg/kg/day group and also for three of the four animals in the highest dose group.

<u>Developmental toxicity</u>
Undiluted test material was applied once daily to the clipped skin on the backs of groups of 12 presumed-pregnant female rats, aged 12-13 weeks, on days 0 to 20 of gestation at doses of 50, 150 and 500 mg/kg/day.
Application sites were alternated (intrascapular and lumbar) to reduce skin irritation. Elizabethan collars were fitted to the rats for 6 hours after each application to reduce oral intake of test material. Any residual test material was wiped from the skin prior to collar removal.
Each animal was observed twice daily for signs of toxicity. Body weights were recorded on days 0, 4, 8, 12, 16 and 20 of gestation and food consumption was recorded for the periods 0-4, 4-8, 8-12, 12-16 and 16-20 days of gestation and days 0 and 4 of lactation.
Each litter was observed daily during days 0 through 4 of lactation for signs of toxicity and mortality.
Each female that was mated was sacrificed and necropsied. Females that delivered a litter were necropsied on day 4 of lactation whilst all other animals were necropsied on presumed day 25 of gestation.
The necropsy included a gross examination of the external body surfaces, orifices and the cervical, thoracic and abdominal viscera.
The number of implantation sites was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide to reveal any implantation sites. If none were found the animal was considered to be non-pregnant.
Dead pups were removed, examined externally and discarded.

Statistical analysis

Body weight and food consumption data
A Bartlett's test was performed to determine if the dose groups had equal variance at the 1% level of significance. If the variances were equal, the testing was done using parametric methods, otherwise non-parametric methods were used.
The parametric procedure was a one-way analysis of variance. Dunnett's test was used to assess significance of differences between test and control. In addition, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.
For the non-parametric procedure a Kruskal-Wallis test was performed. If there were significant differences between the means a Dunn's Summed Rank test was used to determine which treatment groups differed significantly from control. In addition Jonckheere's test for monotonic trend in the dose response was performed.
Test for equal variance (Bartlett) was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% levels of significance.

Reproductive data and litter data
For number of implantation sites, gestation length, total number of pups per litter and number of live pups per litter, normal probability plots of the residuals by treatment group were used to judge whether or not departure from the assumptions of normality and homogenous variance were sufficient to invalidate the usual analysis of variance. If the usual analysis was invalid, a weighted General Linear Model (GLM) analysis was used, where the weight were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.

Result **:** There were no mortalities in the study and there were few treatment-related clinical signs of toxicity. These consisted of yellow, yellow/brown, yellow/orange or red/yellow stained coats for eight animals in the highest dose group. Alopecia was also noted in a few animals in this dose group.
Although there were no significant changes in growth rates in either the 50 or 150 mg/kg/day groups body weights were reduced in the highest dose group when compared to controls. The differences occurred from day 4 of gestation onwards and throughout lactation.
Body weight changes were also significantly less throughout gestation in the highest dose group when compared to controls. However, body weight changes for the highest dose group were significantly higher than controls through lactation. This was attributed to a cessation of exposure to the test material and recovery during this period.

There were no significant differences in absolute or relative food consumption for animals in the lowest dose group throughout gestation when compared to controls.

In the 150 mg/kg/day group absolute food consumption was unaffected but food consumption relative to body weight was significantly higher than that for controls during days 16-20 of gestation.

In the 500 mg/kg/day group Absolute food consumption was higher than controls during lactation. Relative food consumption was higher than controls from day 12 to the end of the study.

Skin irritation occurred in all dose groups during the study as follows:
50 mg/kg/day
Erythema slight to extreme (primarily slight to moderate)
Edema slight to moderate
Eschar slight to extreme (primarily slight to moderate)

150 mg/kg/day
Erythema slight to extreme (primarily slight to moderate)
Edema slight to extreme (primarily slight to moderate)
Eschar slight to extreme (primarily slight to moderate)

500 mg/kg/day
Erythema slight to extreme (primarily moderate to extreme)
Edema slight to extreme (primarily moderate to extreme)
Eschar slight to extreme (primarily moderate to extreme)
Fissuring in two animals for two days

Apart from skin irritation there were no other treatment-related findings at gross necropsy.

Reproduction and litter data are summarized in the following table.

	Dose group (mg/kg/day)			
Parameter	**0**	**50**	**150**	**500**
No. pregnant animals	15	11	10	12
No. that delivered	15	11	10	12
Mean gestation (days)	22.1	22.2	21.8	21.9
Mean No. implantation sites	16.0	15.6	17	16.6
No. litters with live pups	15	11	9	12
Mean No. live pups				
Day 0	14.9	13.5	14.9	15.1
Day 4 survival	97%	99%	99%	70%
Mean Wt. (g) live pups (adjusted)				
Day 0	6.55	6.65	6.10	5.56
Day 4	9.89	10.52	8.41	6.94
Proportion males (adjusted)				
Day 0	0.49	0.49	0.48	0.49
Day 4	0.49	0.48	0.47	0.51

In conclusion:
Developmental effects
At 150 mg/kg/day pup body weights were significantly lower than controls.
At 50 mg/kg/day there were no significant effects on litter data.

Maternal effects
At 50 mg/kg/day there was significant skin irritation in the parental animals.

The no observable adverse effect level for maternal toxicity was, therefore, less than 50 mg/kg/day and for developmental toxicity was 50 mg/kg/day.

Reliability **:** (2) valid with restrictions
The study was well reported but was only a screening study and did not cover fully the developmental toxicity endpoint.

(61)

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Frequency of treatm.	:	Once daily
Duration of test	:	Days 0 through 20 of gestation
Doses	:	125, 250 & 1000 mg/kg/day
Control group	:	Yes
NOAEL maternal tox.	:	< 125 mg/kg bw
NOAEL teratogen.	:	250 mg/kg bw
Year	:	1994
GLP	:	Yes
Test substance	:	Sample F-220 consisted of: 77.7% saturates 22.3% aromatics

Method :

Undiluted test material was applied once daily to the clipped skin on the backs of groups of 15 presumed-pregnant female rats, aged 14-15 weeks, on days 0 to 20 of gestation at doses of 125, 250 mg/kg/day. A further group of 15 presumed-pregnant rats of the same age received test material at a dose level of 1000 mg/kg/day from day 5 through 9 of gestation. A group of 20 presumed-pregnant rats of the same age served as sham treated controls. Application sites were alternated (intrascapular and lumbar) to reduce skin irritation. Elizabethan collars were fitted to the rats for 6 hours after each application to reduce oral intake of test material. Any residual test material was wiped from the skin prior to collar removal.
Each animal was observed twice daily for signs of toxicity. Body weights were recorded on days 0, 4, 8, 12, 16 and 20 of gestation and food consumption was recorded for the periods 0-4, 4-8, 8-12, 12-16 and 16-20 days of gestation and days 0 and 4 of lactation.
Each litter was observed daily during days 0 through 4 of lactation for signs of toxicity and mortality.
Each female that was mated was sacrificed and necropsied. Females that delivered a litter were necropsied on day 4 of lactation whilst all other animals were necropsied on presumed day 25 of gestation.
The necropsy included a gross examination of the external body surfaces, orifices and the cervical, thoracic and abdominal viscera.
The number of implantation sites was recorded. Uteri that appeared non-gravid were placed in 10% ammonium sulfide to reveal any implantation sites. If none were found the animal was considered to be non-pregnant.
Dead pups were removed, examined externally and discarded.

Statistical analysis

<u>Body weight and food consumption data</u>
A Bartlett's test was performed to determine if the dose groups had equal variance at the 1% level of significance. If the variances were equal, the testing was done using parametric methods, otherwise non-parametric methods were used.
The parametric procedure was a one-way analysis of variance. Dunnett's test was used to assess significance of differences between test and control. In addition, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.
For the non-parametric procedure a Kruskal-Wallis test was performed. If there were significant differences between the means a Dunn's Summed Rank test was used to determine which treatment groups differed

significantly from control. In addition Jonckheere's test for monotonic trend in the dose response was performed.
Test for equal variance (Bartlett) was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% levels of significance.

Reproductive data and litter data
For number of implantation sites, gestation length, total number of pups per litter and number of live pups per litter, normal probability plots of the residuals by treatment group were used to judge whether or not departure from the assumptions of normality and homogenous variance were sufficient to invalidate the usual analysis of variance. If the usual analysis was invalid, a weighted General Linear Model (GLM) analysis was used, where the weight were proportional to the reciprocal of the variance. If the usual analysis was valid, the data were analyzed with a non-weighted GLM.

Result **:** There were no mortalities during the study.
The only clinical observation was dermal irritation which was observed in all dose groups as follows:

125 mg/kg/day	slight to extreme (primarily slight to moderate) erythema, edema, eschar and dry skin.
250 mg/kg/day	slight to extreme (primarily moderate to extreme) erythema, eschar and dry skin. Slight to moderate edema. Slight fissuring noted in one female on gestation days 5 through 10.
1000 mg/kg/day	Slight to extreme (primarily moderate to extreme) erythema, edema, eschar and dry skin.

There were no effects on either body weights or body weight changes in the 125 and 250 mg/kg/day groups. However body weight changes were significantly lower than controls for the 1000 mg/kg/day group during the period days 4 through 12 of gestation. From day 16 to day 20 of gestation, body weight changes were higher in the treated group than the controls.

Food consumption was unaffected in the 125 mg/kg/day group.
Minor differences in the 250 mg/kg/day group were not considered to be toxicologically significant.
In the 1000 mg/kg/day group absolute food consumption was lower than controls on gestation days 8 to 12 but was higher than controls days 16 to 20. This was accompanied by similar changes in relative food consumption. The higher values occurred after cessation of exposure to the test material.

The only finding at gross necropsy that was considered to be treatment-related was that of skin irritation.

The litter data are summarized in the following table.

	Dose group (mg/kg/day)			
Parameter	**0**	**125**	**250**	**1000**
No. pregnant animals	16	14	13	14
Mean gestation (days)	22.4	22.4	22.1	22.1
Mean No. implantation sites	16.1	16.8	17.2	17.7
No. litters with live pups				

	16	14	13	14
Mean No. live pups				
Day 0	14.4	14.9	16	15.8
Day 4 survival	93%	92%	93%	91%
Mean Wt. (g) live pups (adjusted)				
Day 0	6.33	6.45	6.36	6.33
Day 4	8.2	8.59	8.56	8.43
Proportion males (adjusted)				
Day 0	0.49	0.52	0.46	0.49
Day 4	0.51	0.51	0.49	0.50

Reliability : (2) valid with restrictions
The study was well reported but was only a screening study and did not cover fully the developmental toxicity endpoint.

(62)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Days 0-19 of gestation
Frequency of treatm. : Daily
Duration of test : Days 0-19 of gestation
Doses : 15, 60, 250 & 500 mg/kg/day
Control group : Yes
Year : 1988
GLP : Yes
Test substance : Coker Light Gas Oil (CLGO) see section 1.1.1.

Method : Coker Light Gas Oil (CLGO) was applied daily to the shorn dorsal skin of groups of ten presumed-pregnant female rats (aged approximately 7 weeks) at the dose levels shown below and for the duration indicated. Since it was believed that inhalation of test material could be a confounding factor a second group of controls (remote controls) were housed in an area in which they could not inhale gas oil that had been applied to other animals.

Groups 1-6 inc. were designated prenatal toxicity groups and were sacrificed on day 20 of gestation. Groups 7 & 8 were postnatal toxicity groups and were sacrificed on day 4 post partum.
The bioavailability group animals were sacrificed on day 13 of gestation.
The study design is outlined as follows:

	Dose level (mg/kg/day)	**Dosing days***
Prenatal toxicity group		
Group 1	0 (Remote sham control)	0-19
Group 2	0 (Proximal sham control)	0-19
Group 3	15	0-19
Group 4	60	0-19
Group 5	250	0-15**
Group 6	500	10-12
Postnatal toxicity group		
Group 7	0 (Sham control)	0-19
Group 8	60	0-19
Bioavailability group (5 rats only)		
Group 9	500	10-12

* denotes days of gestation
** dosing was stopped after day 15 because of severe dermal irritation
Observations were made daily for clinical signs. Body weights and food consumption were recorded regularly throughout the study.

Each female in the prenatal toxicity groups were sacrificed on day 20 of presumed gestation and the thoracic and abdominal cavities were examined grossly.
The uterus and ovaries were removed and examined grossly. The number of corpora lutea per ovary for each rat was recorded. The ovaries of non-pregnant females were examined and then discarded. Uterus weights were also determined. The uterine contents of each pregnant rat were exposed and a record made of the number and location of all implantations.
At necropsy, blood samples were taken from all the animals and the following clinical chemical measurements were made:

Alanine aminotransferase	Glucose
Albumin	Iron
Albumin/globulin ratio	Lactate dehydrogenase
Alkaline phosphatase	Inorganic phosphorus
Aspartate aminotransferase	Potassium
Bilirubin (total)	Sodium
Calcium	Sorbitol dehydrogenase
Chloride	Total protein
Cholesterol	Triglycerides
Creatinine	Urea nitrogen
Globulin	Uric acid

Fetuses were examined and half were preserved for examination of soft tissue abnormalities, the remainder being differentially stained for skeletal examination.

Animals in the post natal groups (parents and offspring) were sacrificed on day 4 post partum. The thoracic and abdominal cavities of each dam were exposed and all organs were examined grossly for abnormalities. The uterus was excised and examined for total number of implantations. Thymus and liver weights were recorded. Although the pups were preserved in fixative, no evaluations were performed.

Animals in the bioavailability group were treated dermally with CLGO containing two radiolabelled markers on gestation days 10, 11 and 12 in metabolism cages. CLGO was applied at a dose of 500 mg/kg and each ml of CLGO contained 15.12 µCi of ^{3}H-BaP and 15.07 µCi of ^{14}C-carbazole.
24 hours after the third application of the labeled CLGO, the animals were killed and blood samples were collected. The amniotic fluid of each of the fetuses was collected. Embryo was separated from the yolk sac and placenta, embryos, amniotic fluid and yolk sacs were pooled for each dam and the weights and volumes were determined.
Maternal tissues collected for radioactivity analysis included: blood, thymus, liver, heart, brain, small intestine, large intestine, kidneys, spleen, stomach, ovaries, urinary bladder, lungs, muscle, retroperitoneal fat, femur and residual carcass. Urine, cage wash and fecal samples were collected from the metabolism cage and analyzed for radioactivity.

Statistical analysis
Maternal biophase data, cesarean section data and fetal data were evaluated statistically by analysis of variance followed by group comparisons using Fisher's exact or Dunnet's test.
Serum chemistry data were analyzed for analysis of variance followed by comparisons using Tukey's test.

For all statistical analyses, differences between control and treated groups were considered to be significant if the probability of the difference being due to chance was less than 5% ($p < 0.05$)

Result **:** The only clinical signs that were considered to be substance-related are those of skin irritation, which ranged from moderate to severe. Erythema, flaking, scabbing and thickening of the skin was observed in all groups exposed to CLGO. In those animals exposed to CLGO for more than 16 days fissuring, eschar and necrosis of the skin was also observed.
Growth rates were normal except for those of the 250 and 500 mg/kg day groups whose body weights were significantly less than controls at day 20 of gestation. The mean maternal weight gains throughout gestation are shown in the following table

	Dose group (mg/kg/day)					
	O(R)[1]	0(P)[2]	15	60	250	500
Day 0-20 weight gain of prenatal groups						
Mean	164	171	153	163	133[bd]	147[c]
SD	18	24	15	18	19	5
N [3]	10	9	10	9	9	10
Day 0-20 weight gain of post natal groups						
Mean		163		135[d]		
SD		14		24		
N		10		9		

[1] Remote control group
[2] Proximal control group
[3] No. of pregnant dams
[b] P <0.01 compared to group 1
[c] P <0.05 compared to group 2
[d] P <0.01 compared to group 2

The body weight changes of controls and treated groups in the post-partum animals were comparable.

Food consumption of the 500 mg/kg/day group was significantly less than controls throughout the study. Those animals in the 250 mg/kg/day group consumed less food than the remote control group during most of gestation up to day 13 after which they were consuming less food than either of the control groups.

At necropsy, thymus weights in the 250 mg/kg/day group were found to be less than the controls but the differences were not statistically significant. The authors did not consider the differences to be treatment related.

None of the following parameters for any CLGO dose group were different from control values:
- No. females aborted
- No. dams with viable fetuses
- No. dams with all resorptions
- Female mortality

No. corpora lutea
No. implantation sites
% preimplantation loss
No. viable fetuses
Litter sizes
Viable male fetuses
Viable female fetuses
Dead fetuses
No. resorptions
No. dams with resorptions

No effects were observed in any of the clinical chemical analyses.
Mean fetal body weights and crown-rump length measurements were unaffected by exposure of the dams to CLGO.
The anomalies observed in the fetuses were considered probably not exposure-related since they either occurred at a low incidence and/or also occurred in the control groups.

Reliability : (1) valid without restriction

(57)

5.9 SPECIFIC INVESTIGATIONS

Type	:	Initiation/promotion assay
Species	:	Mouse
Sex	:	Male
Strain	:	CD-1
Route of admin.	:	Dermal
No. of animals	:	30
Vehicle	:	Undiluted
Year	:	1993
GLP	:	Yes
Test substance	:	DGMK gas oil samples 1,6,7,9 & 11 (See section 1.1.1.)

Method : Five gas oil samples were investigated in this assay.
Groups of 30 male CD-1 mice were used for each treatment.
The following dosing regimes were used.

<u>Assessment of tumor initiating potential</u>
50 µl of each test material was applied undiluted to the shorn dorsal skin of the mice for 5 consecutive days of the first week of the initiating period.
A promoter [TPA (12-0-tetradecanoylphorbol-13-acetate)] was then applied twice a week (50 µg/animal, dissolved in 50 µl acetone) from week 4 to week 28.
As a positive control, DMBA was applied once (50 µg/animal, dissolved in 50 µl acetone) on the first day of the administration period and then followed by treatment with TPA as described above.

<u>Assessment of tumor promoting potential</u>
The animals were initiated by a single dose of DMBA (50 µg/animal dissolved in 50 µl acetone).
From week 4 to week 28, the test materials were applied undiluted twice a week at a dose of 5 µg/animal, dissolved in 50 µl acetone.
As a positive control TPA was applied twice a week instead of the test material.

Negative control animals were initiated with DMBA or acetone, followed by

promotion with acetone or TPA respectively.

During weeks 2 and 3 of the study, the animals were not treated allowing for regression of possible skin alterations that may have occurred during the initiating period.

Body weights were determined once weekly and each week a detailed examination of the skin was performed.
At the end of the study, the animals were assessed grossly, followed by histopathology of the skin and all macroscopic lesions.

Result : Survival of the animals was unaffected by exposure to the gas oil samples. Body weights were only transiently reduced in the positive controls and those treated with sample 7 (predominantly aromatic) and very slightly in animals treated with sample 9 (predominantly saturates).

During the initiation phase, the gas oil samples caused slight to moderate skin irritation consisting of reddening, scale formation, and/or erosions in all the gas oil groups except the group treated with sample 11 (predominantly saturates). Similar skin changes also occurred during the promotion phase.

During the two week recovery period between the initiation and promotion phase the skin changes in all groups were found to be reversible.

There were no treatment-related clinical findings other than skin changes.

The number of animals with neoplastic findings in the treated skin at the end of the study was follows:

Sample	**No. of animals with**			
	SCP*	**KAT****	**SCC*****	
Test for initiating activity				
Predominantly aromatics				
6	4	0	0	
7	14	0	0	
Predominantly saturates				
1	2	0	0	
9	5	0	0	
11	0	0	0	
Negative (Acetone/TPA)		0	0	0
Test for promoting activity				
Predominantly aromatics				
6	0	0	0	
7	7	2	0	
Predominantly saturates				
1	1	0	0	
9	0	0	1	
11	0	0	0	
Negative (DMBA/acetone)		0	0	0
Positive (DMBA/TPA)	30	0	0	

* SCP = Squamous cell papilloma
** KAT = Keratoacanthoma
*** SCC = Squamous cell carcinoma

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Appendix C.

Robust Summary

(Separate document)

ROBUST SUMMARY
OF INFORMATION ON

Substance Group

Distillate Fuels

Summary prepared by American Petroleum Institute

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NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

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A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
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1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : The principal distillate fuels are:
Automotive fuels for diesel engines
- Automotive gas oil (AGO)
- Automotive diesel fuel (DERV)
- Diesel fuel No. 2
- Railroad engine gas oil

Heating oils
- Domestic heating oil
- Industrial heating oil
- Industrial gas oil
- No. 2 fuel oil

Marine fuel
- Distillate marine diesel fuel

The distillate fuel oils listed above are composed of gas oil streams that have been blended to meet specific performance criteria.
The streams that are used may be either straight run or cracked and/or hydrotreated gas oils.

Typical concentration ranges of the refinery stream components for the various fuel types have been published by CONCAWE (1995):

Process stream	**Automotive gas oil**	**Heating oil**	**Distillate marine fuel**
	Concentration range (% v/v)		
Straight-run atmospheric gas oil			
-light	40-100	40-100	40-100
-heavy	0-30	0-40	0-50
Vacuum gas oil	0-10	0-15	0-20
Thermally cracked gas oil	0-20	0-30	0-30
Light catalytically cracked gas oil	0-25	0-35	0-40

The physical chemical characteristics and other descriptors for the samples on which toxicological studies have been conducted are summarized below.

DIESEL FUELS
API Samples:

	API sample No. 79-6	**No.2 DA**
Boiling range (ASTM D-56)	367-675° F	372-656° F
Flash point (ASTM D-56)	142° F	136° F
Wt. Sulphur %	0.19	0.1
Viscosity @ 100° F (ASTM D-445)	2.17 cSt	2.4 cSt

Acid No. (ASTM D-974)	0.0	
Bromine No. (ASTM D-1159)	1.42	0.6
Benzene	80 ppm, v/v	47 ppm, v/v
Hydrocarbon types identified by mass spectrometry were:		
Paraffins	41.7 Wt. %	42.7 Wt. %
Cycloparaffins	30.9 Wt. %	33.4 Wt %
Monocycloparaffins	18.8	23.4
Dicycloparaffins	9.3	8.0
Tricycloparaffins	2.8	2.0
Aromatics	27.4 Wt. %	23.9 Wt. %
Alkylbenzenes	8.4	9.7
Indans & tetralins	5.3	4.8
Dinaphthenobenzenes	1.1	1.3
C11 + naphthalenes	7.6	
Biphenyls etc.	2.5	1.3
Fluorenes etc.	1.1	0.7
Tricyclic aromatics	1.4	0.8

DGMK SAMPLES

3 samples: Nos. 22, 23 & 24.

The source of crude and the blend components used for the manufacture of the diesel fuels are detailed in the report. Other characterization parameters are tabulated below.

Parameter	**DGMK Sample No.**		
	22	**23**	**24**
Density (g/ml @ 15°C)	0.8222	0.8433	0.8337
IBP (°C)	161	151	143
FBP (°C)	349	384	347
Viscosity (mm2/sec @40°C)	2.2	3.25	2.11
Carbon (wt%)	85.94	86.4	86.44
Hydrogen (wt%)	13.85	13.35	13.36
Total nitrogen (mg/l)	46	171	125
Total sulfur (wt%)	0.12	0.17	
(mg/kg)			457
Aromatics (vol.%)	23.4	39.6	28.1
Olefins (vol.%)	0	1.0	1.0
Saturates (vol.%)	76.6	59.4	71.9
Flash point (°C)	66	65	58

HOME HEATING OILS

Three samples were prepared by the American Petroleum Institute. They were composed of straight run gas oil (CAS 64741-44-2 predominantly saturates) to which cracked stock (CAS 64741-59-9, predominantly aromatics) was added as shown in the following table.

	API Sample No.		
	78-3/ 83-01	**78-2/ 83-02**	**78-4/ 83-03**
Concentration of cracked stock	10%	30%	50%
API gravity	39.5	38	36.5
Density @15°C)	0.8267	0.834	0.8414
RI (RI units @20°C)	1.462	1.1675	1.4743
Mol wt. (g/mol)	200	197	195
Total S (wt%)	0.08	0.11	0.14

Total N (ppm/wt)	37	67	114
Total Cl_2 (ppm/wt)	6	6	6
Saturates (vol %)	79.2	73.4	67.8
Olefins (vol %)	2.9	4.5	6.1
Aromatics (vol %)	17.9	22.1	26.1
Boiling range (° F)	364-615	366-629	358-640

1.13 REVIEWS

Memo : IARC

Remark : IARC reviewed the available data on distillate fuels and assessed the strength of evidence that the fuels were a carcinogenic risk to man and animals.
The conclusions of the IARC review were:

Evaluation:
There is inadequate evidence for the carcinogenicity in humans of diesel fuels.

There is limited evidence for the carcinogenicity in experimental animals of marine diesel fuel.

There is limited evidence for the carcinogenicity in experimental animals of fuel oil No. 2.

The overall evaluations were:

Marine diesel fuel is possibly carcinogenic to humans (Group 2B)

Distillate (light) diesel fuels are not classifiable as to their carcinogenicity to humans (Group 3)

Distillate (light) fuel oils are not classifiable as to their carcinogenicity to humans (Group 3)

(25)

2.1 MELTING POINT

Method : ASTM D97
GLP : No data
Test substance : Middle distillate fuels

Remark : By definition, melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 1999). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 1999). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e., crystal formation) and/or viscosity property.

Result :

Sample	Pour Point (°C)	Method	Ref.
Automotive Gas Oil	-5	ASTM D97	CONCAWE 1996
Heating Oil	0	ASTM D97	CONCAWE 1996
Distillate Marine Fuel	-6	ASTM D97	CONCAWE 1996
Diesel Fuel Oil (2002)	-50	ASTM D97	Jokuty et al. 2002
Diesel Fuel Oil (Alaska)	-36	ASTM D97	Jokuty et al. 2002
Diesel Fuel Oil (Canada)	-30	ASTM D97	Jokuty et al. 2002
Diesel Fuel Oil (Southern USA)	-14	ASTM D97	Jokuty et al. 2002

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier and reference database.

(13) (28)

2.2 BOILING POINT

Method : ASTM D86
GLP : No data
Test substance : Middle distillate fuels

Result :

Sample	Boiling Range (°C)	Method	Ref.
Automotive Gas Oil	160-390	ASTM D86	CONCAWE 1996
Heating Oil	160-400	ASTM D86	CONCAWE 1996
Distillate Marine Fuel	170-420	ASTM D86	CONCAWE 1996
Diesel Fuel Oil (Alaska)	141-320	ASTM D86	Jokuty et al. 2002
Diesel Fuel Oil (Canada)	246-388	ASTM D86	Jokuty et al. 2002
Diesel Fuel Oil (USA)	174-352	ASTM D86	Jokuty et al. 2002

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable review dossier and reference database.

(13) (28)

2.4 VAPOUR PRESSURE

Method : Calculated: ASTM D2889
GLP : No data
Test substance : Middle distillate fuels

Remark : Gas oils consist of complex mixtures of various hydrocarbon compounds having diverse structures represented by paraffins, olefins, naphthenes, and aromatics. Molecular weights of these hydrocarbons range between C9 to C30 (CONCAWE 1996; CONCAWE 2001). Although individual hydrocarbon constituents in gas oil exert their own vapor pressures, the vapor pressure of the mixture is the sum of the individual partial pressures of the components. The values given above are considered to be representative of the general category of gas oils.

Result :

Vapor Pressures (kPa)	Method	Ref.
Automotive Gas Oil:		
0.4 (approx.)	ASTM D2889	CONCAWE 1996
Heating Oil:		
0.4 (approx.)	ASTM D2889	CONCAWE 1996
Diesel Fuel Canada:		
2	ASTM D323	Jokuty et al. 2002
Fuel Oil No. 2:		
2	ASTM D323	Jokuty et al. 2002

Reliability : (2) valid with restrictions

(13) (14) (28)

3.5 BIODEGRADATION

Inoculum : Activated sludge
Contact time : 28 day(s)
Method : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year : 2003
GLP : No data
Test substance : Diesel fuel; CAS No. 68334-30-5

Remark : The report states that biodegradation of the test sample achieved 60% by the end of the test, but the classification of readily biodegradable cannot be given to the test sample because it did not achieve the pass level within the 10-day period following 10% biodegradation. However, recent guidance from OECD states that when testing mixtures of structurally related materials such as oils, the 10-day window should not be applied (OECD 2003). Based on the current OECD recommendations, ultra low sulfur diesel is capable of passing a ready biodegradability test.
Although the test substance was not chemically characterized, the report states that ultra low sulfur diesel of the type tested in this study contains 26 - 30% aromatic compounds.

Result : Average biodegradation for duplicate test flasks:

	28 Days	End of 10-d Window
Ultra low sulfur diesel	60	45
Sodium Benzoate (reference)	97	not applicable

Test condition : Activated sludge inoculum for the test was collected from Chester Sewage Works (Welsh Water, Sealand Road, Chester CH1 4LD). The plant treats predominately (approx. 90%) domestic sewage. To reduce background oxygen consumption, the activated sludge was washed then aerated with moist air for approximately 24 hours at the test temperature (20 °C) prior to use. The final inoculum had a concentration of 30 mg dry solids/l.
The diesel fuel was tested in duplicate at a nominal concentration of 30 mg/l, which was equivalent to 102 mg Theoretical Oxygen Demand (ThOD)/l. Test flasks contained 500 ml of inoculated mineral salts medium dosed with 15± 0.5 mg test sample absorbed on a 21 mm Whatman GF/A glass fiber filter. The filter was held on an aluminum foil support during weighing, and both the filter and foil were added to the test vessel. Also included in the experiment were triplicate blank flasks containing inoculated medium and a GF/A filter and aluminum foil and duplicate reference substance flasks containing inoculated medium and 100 mg ThOD/l sodium benzoate (approximately 60 mg/l).
The test was run at 20 °C using a C.E.S. Aerobic Respirometer. The instrument determined the oxygen demand every hour and replenished the oxygen through the electrolysis of copper sulfate. The resolution of the instrument was 0.02 mg oxygen at S.T.P. (standard temperature and pressure).
The extent of biodegradation over 28 days was calculated as the measured biochemical oxygen demand (BOD) expressed as a percentage of the ThOD. The ThOD for diesel fuel was determined previously as 3.4 mg O_2/mg test substance (Battersby 2000). Results were evaluated in light of the pass level for ready biodegradability. The pass level for the OECD 301 F test is = 60% ThOD within the 28-day test period, and it must be attained within 10 days after biodegradation has achieved 10% ThOD.

Reliability : (1) valid without restriction

(9) (12) (30)

Inoculum	:	Activated sludge
Contact time	:	28 day(s)
Method	:	OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year	:	1999
GLP	:	No
Test substance	:	Nigerian Diesel fuel CAS No. 68334-30-5
Remark	:	This diesel fuel stream did not satisfy the test criteria for ready degradability of 60% degradability within 28 days. Although this diesel fuel is not considered 'readily' biodegradable, it is inherently biodegradable since significant degradation did occur, based on EPA guidance for using ready and inherent biodegradability tests (http://www.epa.gov/oppt/exposure/docs/halflife.htm.)
Result	:	Average biodegradation for duplicate test flasks at 28 days: diesel fuel = 57.5%; rapeseed oil = 84.4% (satisfied positive control criteria). The report noted that the oxygen consumption of the blank controls was below 60 mg/l as required by the test guidelines.
Test condition	:	Activated sludge inoculum for the test was collected from Medford Municipal Wastewater Treatment Plant in Medford, NJ. The plant treats predominately domestic sewage. To reduce background oxygen consumption, the activated sludge was aerated for approximately 27 hours prior to use. Sufficient supernatant was decanted to provide a 1% (v/v) inoculum for each respirometry vessel. The sewage inoculum had a microbial density of $1E^4$ colony forming units per ml, as measured using a commercial dip-slide method, and was within the guideline criteria of $1E^7$-$1E^8$ CFU/l. Test flasks contained 990 ml of inoculated mineral salts medium, ca. 25 mg of test substance and 10 ml of sludge supernatant (inoculum). Also included in the experiment were duplicate blank flasks containing inoculated medium and duplicate reference substance flasks containing inoculated medium and 25 mg of low erucic acid rapeseed oil (LEAR). The test was run at 22 °C (±1 °C) using a C.E.S. Aerobic Respirometer for 28 days. The instrument determined the oxygen demand every hour and replenished the oxygen through the electrolysis of copper sulfate. The extent of biodegradation over 28 days was calculated as the measured biochemical oxygen demand (BOD) expressed as a percentage of the ThOD (theoretical oxygen demand).
Reliability	:	(1) valid without restriction

(29)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : Fish Acute Toxicity Test w/ 24 Hr Renewal
Species : Cyprinodon variegatus (Fish, estuary, marine)
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : 57 measured/nominal
Analytical monitoring : Yes
Method : EPA/600/4-90/027 Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.
Year : 1998
GLP : No
Test substance : CAS No. 68476-30-2; No.2 fuel oil

Method : Statistical Method: Trimmed Spearman Karber Method.
Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic, Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result :

Nominal Conc. (mg/l)	BPH (nmoles/mgC)	% Mortality 24hr	48 hr	72 hr	96hr
Control	-----	0	0	0	0
9.9	88	0	0	0	0
32	186	0	0	0	0
60.0	192	0	0	20	60
124	257	0	80	100	100

Based on nominal loading rates:
96-hr LL_{50}= 57 mg/l
95% confidence interval 48-68 mg/l
CBR= Cfiber,critical = 73 µmol/mlPDMS
BPH critical = 202 nmol/mg C

Test condition : Nominal loading rates of 0, 9.9, 32, 60 and 124 mg/l were used to prepare test solutions. Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were natural seawater (20 ppt salinity) obtained from Manasquan Inlet, Manasquan, New Jersey. Test substance was mixed for each individual treatment in dilution water for 24 hours in stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. BPH analysis was performed using SPME

(solid phase microextraction) fibers which had been equilibrated overnight in WAF solutions and quantified using gas chromatography equipped with a flame ionization detector. Fish were 11 days old at initiation of testing, and were obtained from Aquatic Systems, Inc., Fort Collins, CO. Test vessels were 500 ml glass I-Chem jars with Teflon lined caps. Three replicates per treatment and 4 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Water temperature was 24.8 °C. Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 69 foot-candles during full daylight periods. Dissolved oxygen measurements ranged from 5.0 to 7.7 ppm, pH values between 7.6 and 8.3. Duplicate samples were taken of the control on Day 0 and each WAF (from the mixing vessel) prepared on a daily basis. Samples were used to determine bioavailable petroleum hydrocarbons (BPH).
Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight.
Analyses were performed using 100 um PDMS fibers obtained from Supelco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards. BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbuggen et al.

Reliability : (2) Reliable with restrictions. The data reported in this report were obtained as part of a research program to correlate toxicity with Bioavailable Petroleum hydrocarbons (BPH). Thus although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs. All data reported as final were audited by EBSI Quality Assurance unit.

(21) (39)

Type	:	Fish Acute Toxicity Test w/ 24 Hr Renewal
Species	:	Menidia beryllina (Fish, estuary, marine)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LL50	:	3.2 measured/nominal
Analytical monitoring	:	Yes
Method	:	EPA/600/4-90/027 Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.
Year	:	1995
GLP	:	No

Test substance : other TS: CAS No. 68476-30-2; No.2 fuel oil

Method : Statistical Method: Trimmed Spearman Karber Method.

Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum ProductsSETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result : **Nominal**

Conc.	**BPH**		**% Mortality**		
(mg/l)	**(nmoles/mgC)**	**24hr**	**48 hr**	**72 hr**	**96hr**
Control	-----	0	0	0	0
1.25	37.2	0	0	0	0
2.9	50.3	0	7	13	13
5.2	78.1	0	67	100	100
10.0	114.4	13	100	100	100

Based on nominal loading rates:
96-hr LL_{50}= 3.2 mg/l, 95% confidence interval 2.9-3.6 mg/l
CBR= Cfiber,critical = 26 µmol/mlPDMS
BPH critical = 72 nmol/mg C

Test condition : Nominal loading rates of 0, 1.25, 2.9, 5.2 and 10.0 mg/l were used to prepare test solutions. Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were natural seawater (20 ppt salinity) obtained from Manasquan Inlet, Manasquan, New Jersey. Test substance was mixed for each individual treatment in dilution water for 24 hours in stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. BPH analysis was performed using SPME (solid phase microextraction) fibers which had been equilibrated overnight in WAF solutions and quantified using gas chromatography equipped with a flame ionization detector. Fish were 11 days old at initiation of testing, and were obtained from Aquatic Systems, Inc., Fort Collins, CO. Test vessels were 500 ml glass I-Chem jars with teflon lined caps. Three replicates per treatment and 5 organisms per replicate were tested for each treatment and the control.
Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Water temperature was 24.8 °C. Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 69 foot-candles during full daylight periods. Dissolved oxygen measurements ranged from 5.3 to 7.7 ppm, pH values between 7.7 and 8.4.Duplicate

samples were taken of the control on Day 0 and each WAF (from the mixing vessel) prepared on a daily basis. Samples were used to determine bioavailable petroleum hydrocarbons (BPH).
Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight. Analyses were performed using 100 um PDMS fibers obtained from Supelco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards.
BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbuggen et al.

Reliability : (2) Reliable with restrictions.
The data reported in this report were obtained as part of a research program to correlate toxicity with Bioavailable Petroleum hydrocarbons (BPH). Thus although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs. All data reported as final were audited by EBSI Quality Assurance unit.

(19) (39)

Type : Fish Acute Toxicity Test w/ 24 Hr Renewal
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : 6.6 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : No
Test substance : CAS No. 68476-30-2; No.2 fuel oil

Method : Statistical Method: Trimmed Spearman Karber Method, 1977.
Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products, SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME

quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical; also BPH critical) is exceeded. Thus, the Cfiber,critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result :

Nominal Conc. (mg/l)	BPH (nmoles/mgC)	% Mortality 24hr	48 hr	72 hr	96hr
Control	0.2	0	0	0	0
2.3	79.7	0	0	0	0
4.9	102.7	0	0	7	7
9.8	183.4	7	7	50	100
23.2	261.9	43	100	100	100
49.8	314.3	100	100	100	100

Based on nominal loading rates: 96hr LL_{50}= 6.6 mg/l
95% confidence interval, 6.0-7.3 mg/l,
CBR= Cfiber,critical = 56 µmol/mlPDMS
BPH critical = 155 nmol/mg C

Test condition : Nominal loading rates of 0, 2.3, 4.9, 9.8, 23.2 and 49.8 mg/l were used to prepare test solutions. Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. Test substance was mixed for each individual treatment in dilution water for 24 hours in stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. BPH analysis was performed using SPME (solid phase microextraction) fibers which had been equilibrated overnight in WAF solutions and quantified using gas chromatography equipped with a flame ionization detector. Fish were approximately three weeks old at initiation of testing, and were obtained from Thomas Fish company, Anderson, CA. Loading of fish body mass to treatment was 0.193g fish per liter of aqueous solution. Test vessels were 4L glass aspirator bottles with Teflon covered neoprene stoppers. Two replicates per treatment and 7 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Water temperature was 14.1 °C (0.7sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 69 foot-candles during full daylight periods. Dissolved oxygen measurements ranged from 6.9 to 8.2 ppm, pH values between 6.6 and 7.2. Duplicate samples were taken of the control on Day 0 and each WAF (from the mixing vessel) prepared on a daily basis. Samples were used to determine bioavailable petroleum hydrocarbons (BPH). Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight.
Analyses were performed using 100 um PDMS fibers obtained from Supleco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using

a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards.
BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbruggen et al.

Reliability : (2) Reliable with restrictions.
The data reported in this report were obtained as part of a research program to correlate toxicity with Bioavailable Petroleum Hydrocarbons (BPH). Thus although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs. All data reported as final were audited by EBSI Quality Assurance unit.

(20) (39)

Type : Fish Acute Toxicity Test w/ 24 Hr Renewal
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : 57 measured/nominal
Analytical monitoring : Yes
Method : EPA/600/4-90/027 Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms.
Year : 1998
GLP : No
Test substance : CAS No. 68476-30-2; No.2 fuel oil

Method : Statistical Method: Trimmed Spearman Karber Method.
Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products, SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result : **Nominal**

Conc. (mg/l)	BPH (nmoles/mgC)	% Mortality 24hr	48 hr	72 hr	96hr
Control	----	0	0	0	0
41	353	0	0	0	0
78	426.5	33	33	33	100
169	473.1	83	92	100	100
326	573.1	100	100	100	100
612	594.2	58	100	100	100

Based on nominal loading rates: 96-hr LL_{50}= 57mg/l
95% confidence interval 41-78 mg/l
CBR= Cfiber,critical = 140 µmol/mlPDMS,
BPH critical = 388 nmol/mg C

Test condition : Nominal loading rates of 0, 41, 78, 169, 326 and 612 mg/l were used to prepare test solutions. Test solutions were prepared as water accommodated fractions (WAF). The control and dilution water was a laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs. Test substance was mixed for each individual treatment in dilution water for 24 hours in stoppered containers with less than 10% headspace volume. The mixtures were allowed to settle for 1-2 hours prior to drawing off the aqueous solutions for testing. BPH analysis was performed using SPME (solid phase microextraction) fibers which had been equilibrated overnight in WAF solutions and quantified using gas chromatography equipped with a flame ionization detector. Fish were approximately between 6-8 weeks old at initiation of testing, and were obtained from Aquatic Systems, Inc., Fort Collins, CO. Test vessels were 500 ml glass I-Chem jars with teflon lined caps. Three replicates per treatment and 4 organisms per replicate were tested for each treatment and the control. Exposure containers were filled with no headspace and tightly sealed to prevent volatilization. Test solution renewal was performed daily by removing 80% of the test solution and replacing it with fresh WAF solution prepared at least 24 hrs prior to use. Water temperature was 24.9 °C (0.1°C sd). Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 69 foot-candles during full daylight periods. Dissolved oxygen measurements >60% saturation, pH values between 6.4 and 8.8. Duplicate samples were taken of the control on Day 0 and each WAF (from the mixing vessel) prepared on a daily basis.

Samples were used to determine bioavailable petroleum hydrocarbons (BPH). Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight. Analyses were performed using 100 um PDMS fibers obtained from Supelco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards. BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on

2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound.
Calculation of CBR as Cfiber, critical is based on Verbuggen et al.

Reliability : (2) Reliable with restrictions.
The data reported in this report were obtained as part of a research program to correlate toxicity with Bioavailable Petroleum Hydrocarbons (BPH). Thus although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs. All data reported as final were audited by EBSI Quality Assurance unit.

(22) (39)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LL50 : 21 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: Moving average angle method
Result : 96-hr LL_{50} = 21 mg/l, 95% confidence interval of 12 – 40 mg/l based on nominal loading rates.

Mortality at 96 hrs was 1, 0, 0, 6, and 7 in the 0, 3, 10, 30, and 100 mg/l treatments. Only four concentrations were tested which is less than a minimum of five concentrations stated in the guidelines. Water hardness was higher than targeted range of 50 - 250 mg/l as $CaCO_3$. Hardness range of 264 - 288 mg/l as $CaCO_3$ is normal for this laboratory and does not adversely affect the health of the fish. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved hydrocarbons during the test was 26% (range 9 - 42%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 3, 10, 30, 100 mg/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 257 mg/l as $CaCO_3$, hardness 284 mg/l as $CaCO_3$, conductivity 496 µS/cm, pH 7.5). Test substance was mixed in dilution water for ~72 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MS. Mixtures were allowed to settle 1.5 to 2 hrs prior to drawing off the aqueous phase for testing. Test vessels were sealed 11-liter glass aspirators with 7 fish per vessel. Test fish had a mean length of 5.5 cm and a mean weight of 1.2 g. Fingerlings were obtained from Exmoor Trout Farm, North Molton, Devon, U.K. One replicate per treatment and control were used. Test solutions were renewed daily with surviving fish transferred to the freshly prepared WAFs. Test temperature was 15.4 - 16.2 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (8.7 to 9.5 mg/l). pH was 7.0 - 7.8. To monitor the concentration of soluble components in the test solutions, samples were

collected at the beginning and end of each 24-hr period for each of the batches of WAFs prepared during the 96-hr test.

Reliability : (1) valid without restriction

(37)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: Moving average angle method
Result : 96-hr LL_{50} = 65 mg/l, 95% confidence interval of 21 – 290 mg/l based on nominal loading rates.

Mortality at 96 hrs was 0, 0, 0, 5, and 7 in the 0, 1, 10, 100, and 1000 mg/l treatments. Only four concentrations were tested which is less than a minimum of five concentrations stated in the guidelines. Dilution factor of 10 is greater than recommended. Water hardness was higher than targeted range of 50 - 250 mg/l as $CaCO_3$. Hardness range of 270 – 288 mg/l as $CaCO_3$ is normal for this laboratory and does not adversely affect the health of the fish. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved hydrocarbons during the test was 27% (range 10 - 50%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 1, 10, 100, 1000 mg/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 257 mg/l as $CaCO_3$, hardness 284 mg/l as $CaCO_3$, conductivity 496 µmS/cm, pH 7.5). Test substance was mixed in dilution water for ~70 hrs. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MS. Mixtures were allowed to settle 1.5 to 2 hrs prior to drawing off the aqueous phase for testing. Test vessels were sealed 11-liter glass aspirators with 7 fish per vessel. Test fish had a mean length of 5.6 cm and a mean weight of 1.2 g. Fingerlings were obtained from Exmoor Trout Farm, North Molton, Devon, U.K. One replicate per treatment and control were used. Test solutions were renewed daily with surviving fish transferred to the freshly prepared WAFs. Test temperature was 15.0 - 16.0 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (8.8 to 9.2 mg/l). pH was 7.2 - 7.5. To monitor the concentration of soluble components in the test solutions, samples were collected at the beginning and end of each 24-hr period for each of the batches of WAFs prepared during the 96-hr test.

Reliability : (2) Reliable with restrictions.
Dilution factor of 10 used in the definitive test concentrations was several fold higher than guidelines recommendation and resulted in a wide 95% confidence interval for the 96-hr LL_{50}.

(36)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 7.81 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : Yes
Test substance : CAS 68476-30-2, No. 2 Fuel oil (gas oil, unspecified)

Method : Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.

Result : Number of immobilized daphnids after 48 hours were:

Treatment conc. (mg/l)	No. immobilized Daphnids
0 (control)	1
0.625	0
1.25	0
2.5	2
5.0	9
10.0	10

48-hr EL_{50} = 7.81 mg/l based upon nominal loading rate, 95% C.I. range 48-hr EL_{50} = 5.00-10.02 mg/l loading

Source : Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE

Test condition : Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liter of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 0.625, 1.25, 2.5, 5.0 & 10.0 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH was 9.11 at test start and 8.97 at the end (high value may be a concern). Temperature was 20.1-20.5 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old.

Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of

components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : HEL-blend: middle range distillate; 44% Coker (+hydrotreating), 15% FCC (+hydrotreating), 41% straight run; sample ID A-2133

Reliability : (2) Reliable with restriction due to high pH value of purified water, ie, values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 12.99 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : Yes
Test substance : CAS No. 68476-34-6; diesel fuel (Gas oil, unspecified

Method : Statistical method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.

Result : Number of immobilized daphnids after 48 hours were 0, 5, 12, 14, 15, and 17 in the control, 6.25, 12.5, 25, 50 & 100 mg/l treatments, respectively. 48-hr EL_{50} = 12.99 mg/l based upon nominal loading rate,95% C.I. range 48-hr EL_{50} = 7.53-22.41 mg/l loading

Source : Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.

Test condition : Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2liter of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 6.25, 12.5, 25, 50 & 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with10

daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test system solutions ranged from 9.59 to 9.74 (high value may be concern). Temperature was 20.0-20.6 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old.

Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : 10% Kero, 53% hydrotreated LGO, 37% hydrotreated HGO; sample ID A-2145

Reliability : (2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 5.3 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : Yes
Test substance : CAS No. 68476-30-2; No. 2 fuel oil (Gas oil, unspecified)

Method : Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.

Result : Number of immobilized daphnids after 48 hours were 0, 0, 0, 7, 8, and 14 in the control, 0.625, 1.25, 2.5, 5.0 & 10.0 mg/l treatments, respectively.48-hr EL_{50} = 5.30 mg/l based upon nominal loading rate,95% C.I. range 48-hr EL_{50} = 3.22-8.71 mg/l loading

Source : Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.

Test condition : Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and

1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 0.625, 1.25, 2.5, 5.0 & 10.0 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with 10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH was 9.20 through 9.38 (high value may be concern). Temperature was 20.1-20.5 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old.

Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : 13% Kero, 12% LGO, 75% hydrotreated LVGO; sample ID A-2146
Reliability : (2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 6.35 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : Yes
Test substance : CAS No. 68476-34-6; diesel fuel (Gas oil, unspecified)

Method : Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.
Result : Number of immobilized daphnids after 48 hours were 0, 1, 10, 15, 15, and 17 in the control, 1.90, 3.75, 7.5, 15.0 & 30.0 mg/l treatments, respectively. 48-hr EL_{50} = 6.35 mg/l based upon nominal loading rate. 95% C.I. range 48-hr EL_{50} = 4.59-8.78 mg/l loading

Source	:	Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 1.90, 3.75, 7.5, 15.0 & 30.0 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with 10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test solutions ranged from 9.26 to 9.63 (high value may be concern). Temperature was 20.1-20.3 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old. Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.
Test substance	:	13% SR Kero CD3/4; 18% SR LGO CD3/4; 59% hydrotreated LGO CD3; 6% hydrotreated LGO CD4; 3% hydrotreated SGO CD3/4, 2% hydrotreated LCO; 2% hydrotreated Kero; sample ID A-2150
Reliability	:	(2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EL50	:	14.49 measured/nominal
Analytical monitoring	:	Yes

Method	:	OECD Guide-line 202
Year	:	2000
GLP	:	Yes
Test substance	:	CAS No. 68476-30-2; fuel oil (Gas oil, unspecified)
Method	:	Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.
Result	:	Number of immobilized daphnids after 48 hours were 0, 0, 15, 15, 16 and 18 in the control, 1.5, 3.0, 6.00, 12.0 & 24.00 mg/l treatments, respectively. 48-hr EL_{50} = 14.49 mg/l based upon nominal loading rate.95% C.I. range 48-hr EL_{50} = 10.57-19.86 mg/l loading
Source	:	Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 1.5, 3.0, 6.00, 12.0, & 24.00 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with 10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test system solutions ranged from 9.15 to 9.25 (high value may be a concern). Temperature was 19.9-20.2 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old. Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests
Test substance	:	36% hydrotreated LGO CD4; 16% hydrotreated LCO; 2% hydrotreated VGO; 3% hydrotreated VB-GO; 7% hydrotreated CC Kero; 6% SR Kero CD3/4; 23% SR LGO CD3/4; 1% LCO; 6% SRHGO; sample ID A-2151
Reliability	:	(2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5%

was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EL50	:	36.01 measured/nominal
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 202
Year	:	2000
GLP	:	Yes
Test substance	:	CAS No. 68476-34-6; No. 2 diesel fuel (Gas oil, unspecified)
Method	:	Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.
Result	:	Number of immobilized daphnids after 48 hours were 0, 0, 4, 5, 12, and 17 in the control, 6.25, 12.5, 25, 50 & 100 mg/l treatments, respectively. 48-hr EL_{50} = 36.01 mg/L based upon nominal loading rate.;95% C.I. range 48-hr EL_{50} = 25.52-50.79 mg/l loading
Source	:	Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.
Test condition	:	Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 6.25, 12.5, 25, 50 & 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test system solutions ranged from 9.55 to 9.65 (high value may be a concern). Temperature was 20.0-20.6 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old. Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured

during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : DK-blend: middle range distillate; 81% hydrotreating, 19% straight run, sample ID A-2152

Reliability : (2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 9.57 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : Yes
Test substance : CAS No. 68476-34-6; diesel fuel (Gas oil, unspecified)

Method : Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.

Result : Number of immobilized daphnids after 48 hours were 0, 0, 9, 13, 18, and 19 in the control, 3.125, 6.25, 12.5, 25, and 50 mg/l treatments, respectively. Twenty daphnids per treatment were used.
48-hr EL_{50} = 9.57 mg/l based upon nominal loading rate. 95% C.I. range 48-hr EL_{50} = 7.34-12.46 mg/l loading.

Source : Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.

Test condition : Test solutions were prepared as water accommodated fractions (WAF). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 3.125, 6.25, 12.5, 25, & 50 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with 10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test system solutions ranged from 9.41 to 9.67 (high value may be a concern). Temperature was 20.1-20.3 °C. Daphnia magna, STRAUS clone 5

organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old.

Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : 31% Kerosene SR; 27% kerosene HDS; 21% middle oil HDS; 21% middle oil HC; sample ID A-2181

Reliability : (2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 42.43 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2000
GLP : yes
Test substance : CAS No. 68476-30-2; fuel oil (Gas oil, unspecified)

Method : Statistical Method: Probit analysis, Finney. D.J. Stat. Method in Biological Assay. 2nd ed. London, 1984.

Result : Number of immobilized daphnids after 48 hours were 0, 0, 3, 6, 14, and 17 in the control, 7.5, 15, 30, 60, and 120 mg/l treatments, respectively. Twenty daphnids per treatment were used.
48-hr EL_{50} = 42.43 mg/l based upon nominal loading rate, 95% C.I. range 48-hr EL_{50} = 31.59-56.97 mg/l loading.

Source : Study sponsor-DGMK, Deutsche Wissenschaftliche Gesellschaft fur Erdol, Erdgas und Kohle (German Research Organization for Oil, Gas and Coal). Distributed by CONCAWE.

Test condition : Test solutions were prepared as water accommodated fractions (WAFs). Control and dilution water were purified, sterilized drinking water. Purification includes filtration with charcoal, aeration and passage through a lime stone column. This final step may be a factor causing high, but apparently non-toxic pH values for the purified water (>8.5 to 9.88). Equilibration studies were conducted for a diesel and fuel oil at 100 and 1000 mg/l loading. WAFS were prepared in duplicate by mixing the appropriate mass of substance in 2 liters of water for 70 hr in sealed brown glass screw top bottles fitted w/ a port near the bottle bottom for drawing off

WAF samples for GC analysis. An equilibration time of 21 hr was deemed sufficient to achieve maximum aqueous phase saturation of the gas oils. Range finding toxicity studies were conducted for all gas oils at 10 and 100 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Definitive toxicity studies were conducted at 7.5, 15, 30, 60, & 120 mg/l loading, using WAFS which were divided into duplicate aliquots and tested. Test vessels were sealed 200 ml conical flasks with10 daphnids per flask and were completely filled with test solution. Dissolved oxygen was >60% saturation during the study. The pH of the test system solutions ranged from 9.17 to 9.33. Temperature was 19.9-20.2 °C. Daphnia magna, STRAUS clone 5 organisms were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged at least 3 weeks old.

Analytical monitoring confirmed consistency of solubilized components of gas oil in aqueous solution, and was also used during WAF preparation to evaluate equilibration period required for maximum solubility of components. GC/FID analysis of cyclohexane extracts of WAF was based on ISO/DIS 9377-4:1999 method. The individual peak areas of four significant components detected in the WAF extracts were compared to the peak area of the internal standard, n-tetratriacontane. The ratios of each of the four component peak areas to internal standard peak area measured during the equilibration test remained constant after 21 hours of mixing, indicating this was sufficient time to reach maximum solubility for those hydrocarbons with affinity to partition to the aqueous phase. This normalization of peak area was also used to confirm stability of the WAF during the acute range finder and definitive toxicity tests.

Test substance : 7.4% Kerosene SR; 87% middle oil HDS; 5.7% middle oil SR; sample ID A-2182

Reliability : (2) Reliable with restriction due to high pH value of purified water, i.e., values >8.5 for all treatments up to 9.88. However mortality was <10% for control treatments tested concurrently with each of the eight WAFS (5% was observed for one control treatment, no mortality for any other control tests). The high pH may not be of concern, given that the organisms are cultured using this purified water.

(24)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
LL50 : 2 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1998
GLP : No
Test substance : CAS No. 68476-30-2; No. 2 fuel oil

Method : Statistical Method: Trimmed Spearman Karber Method, 1977.
Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products, SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring

dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result :

Nominal Conc. (mg/l)	**BPH nmoles/mgC)**	**% Mortality 24hr**	**48 hr**
Control	2.9	0	0
1.0	42	0	5
1.5	65.2	5	45
3.5	105	20	90
11	144	35	100
28	162	65	100

Based on nominal loading rates:
48hr LL_{50}= 2.0 mg/;l; 95% confidence interval, 1.7-2.5 mg/l,
CBR=Cfiber,critical = 31 µmol/mlPDMS;
BPH critical = 85.3 nmol/mg C

Test condition : Nominal loading rates of 0, 1.0, 1.5, 3.5, 11 and 28.0 mg/l were used to prepare test solutions. Test solutions were prepared as water accommodated fractions (WAF). Individual treatments at each loading rate were prepared. Each treatment was prepared by adding the appropriate amount of test substance (volumetrically using a syringe) to ~2 liters (allowing for optimum surface contact between the test substance and the dilution water) of dilution water in a 2 liter size glass aspirator bottle. Syringe weights were recorded to determine actual loading rates. The solutions were mixed at a vortex of <10% of the static liquid depth of solution. The aspirator bottles were mixed in the dark (covered with dark plastic) for 24 hours on a magnetic stir plate with a Teflon® coated stir bar. After mixing, the treatments were allowed to settle for 1 hour. After settling, the water accommodated fraction (WAF) was removed and added to the test chambers. The control and dilution water reconstituted laboratory blend water prepared from carbon filtered well water and ion exchange softened, reverse osmosis dialyzed well water aged for >24 hrs.. BPH analysis was performed using SPME (solid phase microextraction) fibers which had been equilibrated overnight in WAF solutions and quantified using gas chromatography equipped with a flame ionization detector. Daphnia magna were supplied by testing laboratory; age < 24 hours old; second generation collected from cultures aged sixteen days old. Four replicates per treatment and 5 organisms per replicate were tested for each treatment and the control. Test vessels were 125 ml autoclaved flasks filled with no headspace and tightly sealed to prevent volatilization. Water temperature range was 20.3 to 21.6 °C. Test photoperiod was 16 hrs. light and 8 hr dark, light intensity approx 69 foot-candles during full daylight periods. Dissolved oxygen measurements ranged from 7.4 to 8.0 ppm, pH values between 7.5 and 7.8. Duplicate samples were taken of the control on Day 0 and each WAF (from the mixing vessel). Samples were used to determine bioavailable petroleum hydrocarbons (BPH). Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase

microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight. Analyses were performed using 100 µm PDMS fibers obtained from Supelco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards. BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbruggen et al.

Reliability : (2) Reliable with restrictions.
The data reported in this report were obtained as part of a research program to correlate toxicity with Bioavailable Petroleum Hydrocarbons (BPH). Thus although the work was conducted according to relevant test guidelines and standard operating procedures, the research was not done in strict accordance with GLPs. All data reported as final were audited by EBSI Quality Assurance unit.

(23) (39)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 210 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: Probit analysis
Result : 48-hr EL_{50} = 210 mg/l
95% confidence interval of 160 - 270 mg/l based on nominal loading rates. Numbers of immobilized daphnids after 48 hrs were 0, 0, 0, 0, 5, 11, 15, and 20 in the 0, 10, 22, 46, 100, 220, 460, and 1000 mg/l treatments. No excursions from protocol were noted. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved hydrocarbons during the test was 5.5% (range 0 - 23%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 10, 22, 46, 100, 220, 460, and 1000 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis filtered water following EPA guidelines (hardness 190 mg/l as $CaCO_3$).
Test substance was mixed in dilution water for 68 hrs. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-

MS. Mixtures were allowed to settle 1.5 hrs prior to drawing off the aqueous phase for testing. Test vessels were sealed 150-ml Erlenmeyer flasks with 10 daphnids per vessel. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 14 and 28 days. Two replicates per treatment and control were used. Test temperature was 18.0 - 18.6 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (9.0 to 9.3 mg/l). pH was 7.6 - 8.4. Samples were collected at the beginning and end of the test to monitor the concentration of soluble components in the test solutions.

Reliability : (1) valid without restriction

(34)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EL50 : 68 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: Moving average angle method
Result : 48-hr EL_{50} = 68 mg/l 95% confidence interval of 49 - 94 mg/l based on nominal loading rates.
Numbers of immobilized daphnids after 48 hrs were 0, 0, 0, 0, 20, 20, 20, and 20 in the 0, 10, 22, 46, 100, 220, 460, and 1000 mg/l treatments.
No excursions from protocol were noted. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved hydrocarbons during the test was 15% (range 0 - 23%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 10, 22, 46, 100, 220, 460, and 1000 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis filtered water following EPA guidelines (hardness 162 mg/l as $CaCO_3$).
Test substance was mixed in dilution water for 48 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MS. Mixtures were allowed to settle 1.5 hrs prior to drawing off the aqueous phase for testing. Test vessels were sealed 150-ml Erlenmeyer flasks with 10 daphnids per vessel. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 14 and 28 days. Two replicates per treatment and control were used. Test temperature was 18.1 - 18.9 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (9.1 to 9.2 mg/l). pH was 8.2 - 8.4. Samples were collected at the beginning and end of the test to monitor the concentration of soluble components in the test solutions.

Reliability : (2) valid with restrictions

(35)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)

Unit	:	mg/l
EL50	:	13 measured/nominal
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 202
Year	:	1994
GLP	:	No
Test substance	:	CAS No. 68334-30-5; Diesel (AGO)
Method	:	Statistical Method: Moving average angle method
Result	:	48-hr EL_{50} = 13 mg/l 95% confidence interval of 11 - 15 mg/l based on nominal loading rates. Numbers of immobilized daphnids after 48 hrs were 0, 0, 0, 0, 0, 2, 20, and 20 in the 0, 0.1, 0.3, 1, 3, 10, 30, and 100 mg/l treatments.
Test condition	:	Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 0.1, 0.3, 1, 3, 10, 30, and 100 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis water following EPA guidelines (hardness 180 mg/l as $CaCO_3$). Test substance was mixed in dilution water for 24 hrs in sealed vessels with minimal headspace. Mixtures were allowed to settle 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 140-ml Erlenmeyer flasks (no headspace) with 10 daphnids per vessel. Test daphnids were obtained from the third brood onwards of cultures supplied by the testing laboratory that have been aged <28 days. Two replicates per treatment and control were used. Test temperature was 17.0 - 19.0 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was 9.2 to 9.8 mg/l). pH was 7.9 - 8.1. Samples were collected from the WAFs at the beginning of the test for BTEX analysis by purge and trap GC-FID.
Reliability	:	(2) Reliable with restrictions. Test methods were sufficiently documented and EPA methods were referenced, even though no test guidelines were specified and the test was not conducted under GLP. Chemical analyses conducted were not aimed at monitoring test substance concentrations in the aqueous phase of the WAFs during exposure.

(31)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Analytical monitoring	:	No
Method	:	OECD Guide-line 202
Year	:	2003
GLP	:	No data
Test substance	:	Diesel fuel CAS No. 68334-30-5
Remark	:	Details on the number of exposure levels, concentrations used and any water chemistry measurements made during the test were not provided in the reviewed report. However, the methods of exposure solution preparation and testing (sealed vessels) provide a high level of confidence that the results give a reliable assessment of the toxicity of the test substance to Daphnia magna.
Result	:	48-h EL_{50} = 100 - 300 mg/l No Observed Effect Level (NOEL) = 100 mg/l There was no control mortality during the test.

Test condition	:	Exposure solutions were prepared as water accommodated fractions (WAFs) and were expressed as the test substance loading rate per volume of dilution water (mg/l). WAFs were prepared by adding measured amounts of diesel fuel to measured volumes of dilution water. Two-liter glass flasks containing 2.3 l of dilution water and diesel fuel were sealed, leaving only a small amount of headspace. The contents then were stirred at 150 rpm for approximately 72 hours (±2 hours). After stirring, the contents were allowed to settle for 1-2 hours to permit undissolved material to separate from the WAF. The aqueous phase then was drawn off for use in the test. Control water was subjected to the same preparation regime except no test substance was added to the flasks. Dilution water used in the test was reconstituted fresh water. Test vessels were 150 ml glass Erlenmeyer flasks that were completely filled with the WAF solution. Duplicate flasks were prepared for each WAF treatment and control group. Ten daphnids were added to each flask and the flasks were sealed with a screw cap. The numbers of immobilized daphnids were determined at 24 and 48 hours. Daphnids were considered immobile if they were not observed to swim during a 15 second observation period.
Reliability	:	(2) valid with restrictions Details of the testing methods and results of any water chemistry measurements were not provided in the report. Also, there were no analytical measurements made on the WAF solutions.

(12)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Exposure period	:	72 hour(s)
Unit	:	mg/l
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1998
GLP	:	No
Test substance	:	CAS No. 68476-30-2; Fuel Oil, No. 2
Method	:	Statistical Method: ELb50 values for cell density were determined using linear interpolation method according to the OECD 201 method. The ELr_{50} values for growth rate were calculated based on linear regression of the ln cell density vs time using PROC REGRESSION in SAS.
Remark	:	Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex

mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result :

Nominal Conc. (mg/l)	BPH (nmoles/mgC)	72 h % Inhibition		
		Cell density	Cell Growth	Growth Rate
Control	----------not applicable----------			
0.4	23.9	3.4	2.6	0.4
0.8	26.	-3.4	-2.2	-2.2
1.6	57	48	37	11
3.2	146.7	92	94	66
Based on nominal loading rates:				
72-hr EbL_{50} (mg/l)		1.8	1.9	2.9
95% confidence interval		1.6-2.1	1.5-2.4	could not calculate

CBR =Cfiber, critical = 23 µmol/mlPDMS;
BPH critical = 63 nmol/mg
CNOEC-not calculated

Test condition : Control and dilution water was algal nutrient media (preparation and composition referenced in EPA-600/9-78-018). Nominal loading rates in the definitive test were 0, 0.4, 0.8, 1.6, and 3.2 mg/l. Individual treatment concentrations were prepared as water accommodated fractions (WAF) at each loading rate and used for toxicity testing and GC analysis. Each treatment was prepared by adding the appropriate amount of test substance (volumetrically using a syringe) to 4 liters (allowing for optimum surface contact between the test substance and the dilution water) of dilution water in a 4 liter size glass aspirator bottle. Syringe weights were recorded to determine actual loading rates. The solutions were mixed at a vortex of 20% of the static liquid depth of solution. The treatments were mixed for 24 hours on a magnetic stir plate with a Teflon® coated stir bar. After mixing, the treatments were allowed to settle for 2 hours. After settling, the water accommodated fraction (WAF) was removed from the outlet at the bottom of the vessel. An aliquot of each treatment was removed for pH measurement and SPME extraction. Test vessels were sealed 125 ml Erlenmeyer flasks containing 10 glass beads and filled with test solution. There were five flasks for each test and control treatment. Algal cells were obtained from in-house cultures which were 5 days old at the start of the test. All flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. The appropriate amount of algae was added to each test chamber then the test solutions were added. Once test chambers were filled they were sealed immediately. Test chambers were only opened to remove a sample for cell density determination on Days 1-3. The volume of test solution removed for each evaluation was replaced with spare solution that had been stored in a sealed vessel. A separate spare vessel was prepared for each replacement interval. Test flasks containing glass beads were conditioned by rinsing with the appropriate solution. Test flasks were hand shaken once or twice daily to ensure that the algal cells remain in suspension. Each test flask was placed on a shaker table (100 rpm) for the duration of the study. Cell counts on each test flask were performed daily. Test temperature was 24.8 °C, 0.1° S.D. Test photoperiod was 14 hrs of light, ten hrs dark, with light intensity ranging from 4100 to 4200 Lux. Direct cell counts on each test flask were performed daily using a hemocytometer. Duplicate samples were taken of the control on Day 0 and each WAF (from the mixing vessel) and from a

composite of replicates 1 through 5 at termination. Samples were used to determine bioavailable petroleum hydrocarbons (BPH). Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight.
Analyses were performed using 100 um PDMS fibers obtained from Supleco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards.
BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbruggen et al.

Reliability : (2) Reliable with restriction.
The study data in this report were developed as part of a research program to correlate BPH with toxicity. Thus although the work was conducted according to the relevant guidelines and standard laboratory operating procedures (SOPs), the research work was not done in strict accordance with Good Laboratory Procedures. However, all data in the final report were audited by EBSI QA unit.

(17) (39)

Species : Selenastrum capricornutum (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: EL50 values determined by probit analysis. Williams test used to determine NOELs.

Result : Based on nominal loading rates:
72-hr EL_{50} (biomass) = 25 mg/l
95% confidence interval of 21 - 30 mg/l

72-hr EL_{50} (growth rate) = 78 mg/l
95% confidence interval of 64 - 96 mg/l

72-hr NOEL (biomass) = 3 mg/l
72-hr NOEL (growth rate) = 10 mg/l.

Nominal Conc. (mg/l)	72 h % Inhibition	72 h Mean Cell Conc. (million cells/ml)
Control	n/a	0.14
1.0	-21	0.17
3.0	-36	0.19
10	29	0.10
30	61	0.055
100	91	0.013
300	94	0.0087
1000	95	0.0074

n/a - Not applicable
The pH increased by more than one unit (1.1 units) during the test as a result of good culture growth and could not be avoided. Temperature range in the incubator was outside the preferred range of 21 - 25 °C. The out-of-range readings were recorded in the first four hours of the test probably due to temperature fluctuations associated with opening and closing the incubator. These deviations were not sufficient to invalidate the study. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved hydrocarbons during the test was 14% (range 0 - 40%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 1, 3, 10, 30, 100, 300, and 1000 mg/l. Control and dilution water was algal nutrient medium prepared according to EPA guidelines except that boric acid was present at 105 µg/l and sodium bicarbonate at 50 mg/l.
Test substance was mixed with dilution water for 72 hrs, and the mixture was allowed to settle for 1.5 - 2 hrs prior to drawing off the aqueous phase for testing. Test vessels were sealed 287 ml Erlenmeyer flasks filled with test solution. There were four flasks for each treatment and seven control flasks. Three of the four treatment and six of the seven control flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Uninoculated flasks were used to determine particle counts without algal cells using a Coulter Multisizer. Flasks were incubated in a cooled orbital (100 cycles/min) incubator. Biomass was calculated as area under the growth curve. Test temperature was 19.5 - 27.6 °C. Lighting was continuous at ~5100 lux. The pH ranged from 8.3 - 8.7 at test initiation and 7.9 - 9.5 at test termination. Samples were collected at the beginning and end of the test to monitor the concentration of soluble components in the test solutions.

Reliability : (1) valid without restriction

(32)

Species : Selenastrum capricornutum (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 68334-30-5; Gas oil

Method : Statistical Method: EL_{50} values determined by probit

analysis. Williams test used to determine NOELs.

Result : Based on nominal loading rates:
72-hr EL_{50} (biomass) = 10 mg/l 95% confidence interval of 8.4 - 12 mg/l

72-hr EL_{50} (growth rate) = 22 mg/l 95% confidence interval of 19 - 26 mg/l

72-hr NOEL (biomass) = 1 mg/l
72-hr NOEL (growth rate) = 3 mg/l.

Nominal Conc. (mg/l)	72 h % Inhibition	72 h Mean Cell Conc. (million cells/ml)
Control	n/a	0.17
1.0	0	0.17
3.0	24	0.13
10	53	0.08
30	91	0.016

n/a - Not applicable

Only four concentrations were tested which is less than a minimum of five concentrations stated in the guidelines.
Analytical method used was gas chromatography-mass spectrometry.
Mean reduction in the concentration of dissolved hydrocarbons during the test was 14% (range 5 - 25%).

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 1, 3, 10 and 30 mg/l. Control and dilution water was algal nutrient medium prepared according to EPA guidelines except that boric acid was present at 105 µg/l and sodium bicarbonate at 50 mg/l. Test substance was mixed with dilution water for ~48 hrs, and the mixture was allowed to settle for 1.5 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 287 ml Erlenmeyer flasks filled with test solution. There were four flasks for each treatment and seven control flasks. Three of the four treatment and six of the seven control flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Uninoculated flasks were used to determine particle counts without algal cells using a Coulter Multisizer. Flasks were incubated in a cooled orbital (100 cycles/min) incubator. Biomass was calculated as area under the growth curve. Test temperature was 21.3 - 23.6 °C. Lighting was continuous at ~3720 lux. The pH ranged from 8.0 - 8.6 at test initiation and 8.5 - 9.2 at test termination. Samples were collected at concentration of soluble components in the test solutions.

Reliability : (1) valid without restriction

(33)

Species : Skeletonema costatum (Algae)
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
Method : other: ASTM Annual Book of Standards, Volume 11.04, Standard Guide for Conducting Static 96 hour Toxicity Tests with Macroalgae, E1218-90
Year : 1995
GLP : No
Test substance : CAS No. 68476-30-2; Fuel Oil, No. 2

Method : Statistical Method: ELb_{50} values for cell density were determined using linear interpolation method according to the OECD 201 method. The ELr_{50} values for growth rate were calculated based on linear regression of the ln cell density vs time using PROC REGRESSION in SAS.

Remark : Data have been developed which allow the quantification of complex hydrocarbons in water on a molar basis using SPME. (Parkerton, T.F., et al., Biomimetic Extraction as a Cost-Effective Analytical Tool for Determining the Aquatic Toxicity Hazard of Complex Petroleum Products SETAC Europe, 2001). The measured molar concentrations reflect those portions that are potentially bioaccumulative in aquatic and terrestrial species. SPME is a surrogate for organism lipid and since it is measuring dissolved, unbound hydrocarbon, the BPH concentration on the fiber is a direct measure of potential toxicity. Data developed at EMBSI comparing SPME molar concentrations of fuel oil in WAFs versus fuel oil toxicity assessed with a wide variety of aquatic species indicates that SPME quantification of bioavailable petroleum hydrocarbons (BPH) in fuel oil correlates well with observed aquatic toxicity. The total molar sum of components that partition to the fiber from the aqueous phase of a complex mixture in a lethal loading test "mimics" the total body residue in an aquatic test organism. Acute toxicity is predicted once a critical threshold on the SPME fiber (Cfiber,critical, also BPH critical) is exceeded. Thus, the Cfiber, critical provides a simple analytical measure that is comparable to the narcosis-based critical body residue (CBR) for a given test organism/endpoint.

Result :

Nominal Conc. (mg/l)	BPH (nmoles/mgC)	72 h % Inhibition Cell density	Cell Growth	Growth Rate
Control	0.3	---not applicable----		-
0.4	15.1	-6.3	12	-0.67
1.0	30.4	-19	11	1.0
2.4	77.4	58	43	32
6.0	166.7	92	83	92
15	207.3	98	83	100
Based on nominal loading rates:				
72-hr EL_{50} (mg/l)		2.2	5.8	2.2
95% confidence interval		2.0-2.4	could not calculate	0.5-16.2

Test condition : Control and dilution water was enriched seawater nutrient medium with 100 mg/l sodium. Nominal loading rates in the definitive test were 0, 0.4, 1.0, 2.4, 6.0 and 15 mg/l.
Individual treatment concentrations were prepared as water accommodated fractions (WAF) at each loading rate and used for toxicity testing and GC analysis. Each treatment was prepared by adding the appropriate amount of test substance (volumetrically using a syringe) to 2 liters (allowing for optimum surface contact between the test substance and the dilution water) of dilution water in a 2 liter size glass aspirator bottle. Syringe weights were recorded to determine actual loading rates. The solutions were mixed at a vortex of 20% of the static liquid depth of solution. The treatments were mixed for 24 hours on a magnetic stir plate with a Teflon® coated stir bar. After mixing, the treatments were allowed to settle for 2 hours. After settling, the water accommodated fraction (WAF) was removed from the outlet at the bottom of the vessel. An aliquot of each treatment was removed for pH measurement and SPME extraction. Test vessels were sealed 125 ml Erlenmeyer flasks filled with test solution. There were four flasks for each test and control treatment. All flasks were inoculated with algal cells to yield an initial concentration of 10,000 cells/ml.

Algal cells were obtained from in-house cultures which were 5 days old at the start of the test. The appropriate amount of algae was added to each test chamber then the test solutions were added. Once test chambers were filled they were sealed immediately. Test chambers were only opened to remove a sample for cell density determination on Days 1-3. The volume of test solution removed for each evaluation was replaced with spare solution that had been stored in a sealed vessel. A separate spare vessel was prepared for each replacement interval.
Test flasks were hand shaken once or twice daily to ensure that the algal cells remained in suspension. Test temperature was 20.4 °C, 0.3° S.D. Test photoperiod was 14 hrs of light, ten hrs dark, with light intensity ranging from 4100 to 4200 Lux. Direct cell counts on each test flask were performed daily using a hemocytometer. Duplicate samples were taken of each WAF (from the mixing vessel) and the control on Day 0 and from a composite of replicates 1 through 5 at termination. Samples were used to determine bioavailable petroleum hydrocarbons (BPH). Measurement of dissolved, bioavailable hydrocarbon was performed using solid phase microextraction (SPME) fiber analysis by GC/FID. BPH samples were mixed (minimal headspace) in vessels containing the SPME fibers overnight. Analyses were performed using 100 µm PDMS fibers obtained from Supleco. 125 ml glass flasks fitted with ground glass stoppers and equipped with stir bars were filled with WAF samples. SPME fibers were inserted through a small hole drilled in the center of the glass stopper. The total moles of hydrocarbons that partition to the fibers were quantified using a gas chromatograph equipped with a flame ionization detector (GC-FID). FID detector response was translated into molar hydrocarbon concentration (BPH as nmoles/mg C) using the molar response based on a number of aromatic standards.
BPH values are reported as nanomoles of quantified hydrocarbon per mg of carbon based on carbon content of polydimethylsiloxane (PDMS) fiber coating. Critical SPME fiber concentration (Cfiber, critical), comparable to critical total body residue (CBR, on a lipid basis) is calculated from BPH results and is reported in units of µmoles/ml of PDMS. Quantitation was based on 2, 3 dimethyl napthalene since a wide variety of hydrocarbons exhibited a relative molar response of within a factor of two when normalized to this compound. Calculation of CBR as Cfiber, critical is based on Verbuggen et al.

Reliability : (2) Reliable with restriction.
The study data in this report were developed as part of a research program to correlate BPH with toxicity. Thus although the work was conducted according to the relevant guidelines and standard laboratory operating procedures (SOPs), the research work was not done in strict accordance with Good Laboratory Procedures. However, all data in the final report were audited by EBSI QA unit.

(18) (39)

Species : other algae: Raphidocelis subcapitata
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : No
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 2003
GLP : No data
Test substance : Diesel fuel CAS No. 68334-30-5

Remark : Details on the number of exposure levels, concentrations used and any

water chemistry measurements made during the test were not provided in the reviewed report. However, the methods of exposure solution preparation and testing (sealed vessels) provides a high level of confidence that the results give a reliable assessment of the toxicity of the test substance to R. subcapitata.

Result : 72-hour Area Under the Growth Curve
EbL_{50} = 10 - 22 mg/l
No Observed Effect Level = <1 mg/l

72-hour Average Specific Growth Rate
ErL_{50} = 22 - 46 mg/l
No Observed Effect Level = <1 mg/l

The growth in the control flasks exceeded a factor of 16 within the 3 days of the test and therefore achieved the validity criteria of the test guidelines.

Test condition : Exposure solutions were prepared as water accommodated fractions (WAFs) and were expressed as the test substance loading rate per volume of dilution water (mg/l). WAFs were prepared by adding measured amounts of diesel fuel to measured volumes of algal growth medium. Two-liter glass flasks containing 2.3 l of growth medium and diesel fuel were sealed, leaving only a small amount of headspace. The contents then were stirred at 150 rpm for approximately 72 hours (±2 hours). After stirring, the contents were allowed to settle for 1-2 hours to permit undissolved material to separate from the WAF. The aqueous phase then was drawn off for use in the test. Algal growth medium for the control group was subjected to the same preparation regime except no test substance was added to the flasks.

The sealed 72-h growth inhibition test was carried out in 287 ml full-volume Erlenmeyer flasks. To each flask was added 5.7 ml of a 2.5 g/l solution of sodium bicarbonate followed by the WAF solution or control medium to make up 287 ml. Four replicate flasks were used for each WAF treatment while seven replicate flasks were used for the control group. Three out of each set of four flasks containing WAF and six out of the seven control flasks were inoculated with sufficient R. subcapitata to give an initial concentration of approximately 5000 cells/ml. The uninoculated flasks were used to determine background particle counts in the absence of algal cells. The flasks were sealed and randomly placed in cooled orbital incubators (approximately 100 cycles/min at a nominal
23±2 °C) under constant illumination. Cell counts were made using a Coulter Multisizer on samples taken from each flask at the start of the test and then at approximately 24 h intervals. Results were evaluated using growth measures based on area under the growth curve and average specific growth rate as recommended in the OECD test guidelines. The subscripts EbL_{50} and ErL_{50} were used to differentiate between measures of effect on the basis of area under the growth curve and growth rate.

Reliability : (2) valid with restrictions
Details of the testing methods and results of any water chemistry measurements were not provided in the report. Also, there was no analytical measurements made on the WAF solutions.

(12)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	9 ml/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	10
Vehicle	:	Undiluted
Year	:	1980
GLP	:	No data
Test substance	:	Diesel fuel, sample API 79-6 (See section 1.1.1.)

Method :

Food was withheld from the rats overnight prior to dosing.
A single dose of test material was given by gavage to groups of 5 male and 5 female rats at dose levels of 2.5, 5.0, 10, 15 & 20 ml/kg.
Daily observations were made for death or signs of toxicity during the 14 day duration of the study. Body weights were recorded at the start and on the 7th and 14th day of the study.
A gross necropsy was performed on all animals that died during the study and on all survivors that were sacrificed on day 14.

Result : Mortality rates were as follows:

Dose group (ml/kg)	Mortality (%)
2.5	12.5
5	20
10	70
15	40
20	90

Signs of toxicity were the same for all dose groups and increased in severity with increasing dose. The signs included oily urine stains and oily diarrhea. The urine and feces stayed on the fur and caused hair loss, irritation, redness and sores on the affected skin. In many animal open sores were observed on the skin surrounding the anus. Blood around the eyes, nose and mouth was also common. Other signs noted included lethargy and pus or blood at the urinary orifice. Observations at gross necropsy were similar for each dose group.
Almost all animals that died before the 14th day had intestinal damage. The intestines and often the stomach were hemorrhagic, sometimes observed with blood. The intestinal walls were thin. Test material was found in the cecum for many days after dosing and a few rats had white spots on their cecums and an increased amount of gas was noted in the intestinal tract.
Animals surviving 14 days had fewer abnormalities, all minor in nature. These included enlarged Peyer's patches on the intestine, an indication that some irritation had occurred.

Conclusion : The oral median lethal dose was 9.0 ml/kg with a 95% confidence interval of 5.58 to 14.51 ml/kg.

Reliability : (1) valid without restriction
Although the study was conducted before Good Laboratory Practices were published, the study appears to have been conducted using appropriate methods and to have been fully reported.

(7)

Type : LD50
Species : Rat
Test substance : Home heating oil containing cracked stocks (See section 1.1.1.)

Result : API reported acute oral toxicity studies on three samples of home heating oil prepared from blends of straight run and cracked stocks.
The LD_{50} values are shown below

Sample	Saturates content (%)	LD_{50} (ml/kg)	API report number
API 78-4	67.8	21.2	API 27-32068
API 78-2	73.4	19	API 27-32771
API 78-3	79.2	14.5	API 27-32773

Reliability : (1) valid without restriction
Although the studies were conducted before Good Laboratory Practices were published, they appear to have been conducted using appropriate methods and to have been fully reported.

(4) (5) (6)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 5 ml/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 8
Vehicle : Undiluted
Year : 1980
GLP : No data
Test substance : Diesel fuel, sample API 79-6 (See section 1.1.1.)

Method : 24 hours prior to testing 5 male and 5 female rabbits were weighed, and their dorsal skin shaved. The shaved skin of 2 males and 2 females was abraded with a hypodermic needle, sufficient to cut the stratum corneum but not so deep as to disturb the dermis or cause bleeding. A single 5 ml/kg dose (calculated on day 0 body weights) of test material was applied to a gauze sponge which was then placed on the skin test site. The gauze was covered by an occlusive dressing.
After a 24 hour exposure period the dressings were removed and any residual test material was removed from the skin by wiping with gauze sponges. Throughout the study the animals were examined for behavioral reactions and other signs of toxicity and the skin test sites were examined for local reactions. Body weights were recorded at the beginning of the study and again after 7 and 14 days. Surviving animals were sacrificed on the 14th day and were subjected to a gross necropsy.

Result : There were no clinical signs of toxicity and the animals did not lose weight during the study.
Erythema followed by drying and flaking of the skin was noted at the test site in all rabbits. At gross necropsy congested kidneys were noted in 4 rabbits, two animals had hemorrhages in the trachea and one rabbit had a congested liver.

Conclusion : The acute dermal LD_{50} of the test material was greater than 5 ml/kg. Exposure to the test material did not cause any compound-related changes.

Reliability : (1) valid without restriction

Although the study was conducted before Good Laboratory Practices were published, the study appears to have been conducted using appropriate methods and to have been fully reported.

(7)

Type : LD_{50}
Test substance : Home heating oil containing cracked stock (See section 1.1.1.)

Result : API reported acute dermal toxicity studies on three samples of home heating oil prepared from blends of straight run and cracked stocks. The LD_{50} values are shown below

Sample	Saturates content (%)	LD50 ml/kg	API report no.
API 78-4	67.8	>5	API 27-32068
API 78-2	73.4	>5	API 27-32771
API 78-3	79.2	>5	API 27-32773

Reliability : (1) valid without restriction
Although the studies were conducted before Good Laboratory Practices were published, they appear to have been conducted using appropriate methods and to have been fully reported.

(4) (5) (6)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
PDII : 6.81
Method : Draize Test
Year : 1980
GLP : No data
Test substance : Diesel fuel, sample API 79-6 (See section 1.1.1.)

Method : Four test sites approximately one inch square were prepared on each of 3 male and 3 female rabbits by clipping. Two of the sites on each animal were abraded with a hypodermic needle, sufficient to cut the stratum corneum, but not deep enough to either disturb the dermis or cause bleeding. For each test site, 0.5 ml of test material was applied to a gauze patch which was then placed on the skin. The gauze patches were secured in place with a bandage and an occlusive covering.
24 hours after the patches were applied they were removed and any surplus test material was removed from the skin by wiping with a gauze sponge.
24 hours after patch removal the skin at the test site was examined and scored for erythema and edema (Draize scale).

Result : At 24 hours the skin of all rabbits was very irritated. Blisters had formed and some of these had opened.
At 72 hours the sites were still very irritated and open sores were observed in 2 rabbits.
At day 7 scabs had formed at most test sites
At day 14 the skin was healing but was still dry and flaky and there had been no hair growth.

Average scores for erythema and edema are listed below.

	Exposure time (Hrs)	Average score
Erythema		
Intact skin	24	4.0
	72	3.83
Abraded skin	24	4.0
	72	3.83
Edema		
Intact skin	24	3.0
	72	2.92
Abraded skin	24	3.0
	72	2.67

Conclusion : Extremely irritating
Reliability : (1) valid without restriction
Although the study was conducted before Good Laboratory Practices were published, the study appears to have been conducted using appropriate methods and to have been fully reported.

(7)

Species : Rabbit
Test substance : Home heating oil containing cracked stocks (See section 1.1.1.)

Result : API reported skin irritation studies on three samples of home heating oil prepared from blends of straight run and cracked stocks.
The primary irritation indices are shown below

Sample	Saturates content (%)	Irritation index	API report no.
API 78-4	67.8	3.83	27-32068
API 78-2	73.4	3.37	27-32771
API 78-3	79.2	3.98	27-32773

Reliability : (1) valid without restriction
Although the studies were conducted before Good Laboratory Practices were published, they appear to have been conducted using appropriate methods and to have been fully reported.

(4) (5) (6)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : Rinsed after (see exposure time)
Number of animals : 9
Result : Not irritating
Method : Draize Test
Year : 1980
GLP : No data
Test substance : Diesel fuel, sample API 79-6 (See section 1.1.1.)

Method : Four male and 5 female rabbits were used in this study.
0.1 ml of undiluted test material was placed on the everted lower eyelid of the right eye of each rabbit. The left eyes were not treated and served as controls. 30 seconds after application, the eyes of 3 rabbits (2 females, one male) were flushed for one minute with warm distilled water.

Scoring for ocular lesions was done at 24, 48 and 72 hours after treatment using the Draize scale. Fluorescein was used to aid evaluation at the 24 hour reading.

Result : All rabbits scored zero at every observation period.
Those rabbits that had their eyes rinsed appeared no different to the unrinsed group.

Reliability : (2) valid with restrictions
Although the study was conducted before Good Laboratory Practices were published, the study appears to have been conducted using appropriate methods and to have been fully reported.

(7)

Species : Rabbit

Result : API reported eye irritation studies on three samples of home heating oil prepared from blends of straight run and cracked stocks.
The primary irritation indices are shown below

Sample	Saturates content (%)	Eye Irritation Index rinsed eye	Unrinsed eye	API Report No.
API 78-4	67.8	0	0	27-32068
API 78-2	73.4	0.7	0.7	27-32771
API 78-3	79.2	0	1.33	27-32773

Reliability : (1) valid without restriction
Although the studies were conducted before Good Laboratory Practices were published, they appear to have been conducted using appropriate methods and to have been fully reported.

(4) (5) (6)

5.3 SENSITIZATION

Type : Patch-Test
Species : guinea pig
Concentration : 1st: Induction undiluted occlusive epicutaneous
2nd: Challenge undiluted occlusive epicutaneous
Number of animals : 10
Result : Not sensitizing
Year : 1980
GLP : No data
Test substance : Diesel fuel, sample API 79-6 (See section 1.1.1.)

Method : 0.5 ml of undiluted test material was applied to a one inch square gauze patch which was then placed on the shorn, depilated skin of 10 male guinea pigs. The applied material was covered with an occlusive dressing which was left in place for 6 hours. This procedure was repeated at the same test site, three times a week for 3 weeks. After the tenth application, the animals were allowed to rest for two weeks without any treatment. After the two weeks rest period 0.5 ml of the test material was applied to the shorn skin of each animal, but on this occasion to a virgin site on the animal's other side.

This procedure was also followed with a group of 10 positive control animals in which chlorodinitrobenzene (0.05% in ethanol) was applied.

24 hours after each application of test or control material the treated site was evaluated and scored for erythema and

Result : The average scores for erythema and edema during the induction phase and after challenge are tabulated below.

	Test material	Positive control
Induction		
Erythema	1.3	1.3
Edema	0.3	0.3
Challenge		
Erythema	1.3	1.9
Edema	0.3	0.7

The authors concluded that the test material was not sensitizing since the differences between induction and challenge scores were not statistically significant.

Reliability : (2) valid with restrictions
The response to the positive control challenge was not remarkable. It is doubtful therefore, that the assay used is sufficiently sensitive.
Although the study was conducted before Good Laboratory Practices were published, the study appears to have been conducted using appropriate methods and to have been fully reported.

(7)

5.4 REPEATED DOSE TOXICITY

Type :
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : Dermal
Exposure period : Four weeks
Frequency of treatm. : Daily, five days per week for four weeks
Doses : 0.5, 2 & 5 ml/kg/day
Control group : Yes
Year : 1986
GLP : Yes
Test substance : Diesel fuel No. 2
Sample F-75-01 is a diesel fuel No. 2
Its composition is:
Saturates 60.4%
Aromatics 39.6%

Method : Three groups of ten male and ten female young adult Sprague-Dawley rats were administered test material to the shorn dorsal skin once daily, five days per week for four weeks at doses of 0.5, 2 or 5 ml/kg/day.
The applied material was covered with an occlusive patch for six hours.
A further group of ten male and ten females served as sham-treated controls.
The animals were observed twice daily for clinical signs of toxicity. Dermal irritation at the application site was assessed daily prior to the next application of test material. An assessment of dermal irritation was also made 24 hours after the final application, just prior to necropsy.
Body weights were recorded three times weekly and just prior to necropsy.
At necropsy, a blood sample was taken for the following hematological and

clinical chemical determinations:

Hematology	Clinical chemistry
Erythrocyte count	Glucose
Total leukocyte count	Blood urea nitrogen
Differential leukocyte count	Alkaline phosphatase
Hemoglobin	SGOT
Hematocrit	SGPT
	Total protein

The following organs were weighed:
Liver
Kidneys (both)
Testes (both)/Ovaries (both)
Brain
Spleen

The following tissues were taken, were fixed and prepared for microscopic examination.
Spleen
Liver
Kidneys (both)
testes/ovaries
Brain (cerebrum, cerebellum, pons)
Skin (treated and untreated)
Bone marrow (smear)
Gross lesions.
The following tissues were removed and preserved but were not examined.

Salivary glands	Thyroid glands
Thymus	Adrenal glands
Trachea	Stomach
Esophagus	Duodenum
Cervical lymph node	Jejunum
Heart	Ileum
Colon	Lungs

Histopathology was done on the sham treated control group and the high dose group animals only.

Statistical methods
Body weights, clinical chemistry, terminal body weights, absolute and relative organ weights were examined using Dunnet's t-test at the 5% probability level.

Result **:** There were no mortalities or any other treatment-related clinical signs of toxicity during the study with the exception of an effect on body weights and the occurrence of skin irritation.

After the second week of the study, the body weights of the mid and high dose males were less than those of the controls and this difference persisted throughout the study. At the end of the study the weight gains of the mid and high dose males were 43 and 13% respectively of those of the controls.

Skin irritation occurred at all dose levels as follows:
Low dose (0.5 ml/kg/day)
Moderate erythema with slight edema which tended to be progressive over the first two weeks. The irritation plateaued after the second week. After the weekends, when there was no dosing, the skin showed some recovery.

Eschar and fissuring peaked during the second week and declined in intensity over the last two weeks of the study.

Mid dose (2 ml/kg/day)
Severe irritation which was progressive during the first week. The level of irritation plateaued during the second week and remained constant thereafter. Eschar and fissuring were evident during the first week and became progressively worse with time. There was evidence of recovery during the fourth week.

High dose (5 ml/kg/day)
Severe irritation which progressively worsened during the first week but plateaued during the second week. The lesions included moderate erythema, mild edema including ulceration, eschar and fissuring. There was some evidence of recovery during the last week of the study in that the degree of irritation appeared to diminish in intensity.

There were some differences in organ/body weight ratios recorded but these were not considered to be a direct effect of treatment but rather as a consequence of reduced body weights of the animals.

Although there were some differences in some clinical chemistry and hematological parameters, they were not dose-related and were not, therefore, considered to be treatment-related.

At gross necropsy, the only treatment-related finding was the presence of skin irritation which included dry skin and scab formation and was consistent with the clinical findings throughout the study.

Histological examination of the tissues taken from rat in the high dose group did not reveal any treatment-related effects other than skin irritation at the site of application. These included acanthosis, hyperkeratosis, dermal fibrosis, epidermal crusting, dermal inflammation and ulceration.

In conclusion the primary effect of dermal exposure to test material was skin irritation at all dose levels, with more irritation occurring at the highest two dose levels.
In the highest two dose groups body weight gains were also reduced.
There were no other biologically significant findings.

Reliability : (1) valid without restriction

(38)

Type	:	Sub-acute
Species	:	Rabbit
Sex	:	Male/female
Strain	:	New Zealand white
Route of admin.	:	Dermal
Exposure period	:	3 weeks
Frequency of treatm.	:	5 days each week for 3 weeks
Doses	:	0.2, 0.67 & 2.0 g/kg
Control group	:	Yes
Year	:	1984
GLP	:	Yes
Test substance	:	Diesel fuel LF-7765 RI

Method : The test material was applied undiluted to the shorn skin of groups of 10

male and 10 female rabbits at dose levels of 0.2, 0.67 and 2.0 g/kg/day. Dose was adjusted by altering the dose volume. The treated skin site was not covered after application of test material and ingestion was prevented by fitting collars to the animals. Two groups of 10 male and 10 females served as untreated controls and sham-treated controls. Animals were dosed once each day, 5 days each week for 3 weeks. All rabbits were observed twice daily for morbidity and mortality except on non-dosing days when the observations were made only once per day. Dead rabbits were removed for necropsy and animals not expected to survive were sacrificed and necropsied. Body weights were recorded 24 hours prior to the study, weekly during the study and immediately prior to sacrifice at termination. The skin at the application site was examined 24 hours after the first application of test material and weekly thereafter. The skin reactions were scored according the Draize scale.
Blood samples were obtained by cardiac puncture from each rabbit (after 18-24 hours fasting), and from each surviving animal at the end of the study. The blood samples were analyzed for a range of clinical chemical and hematological parameters.
All rabbits whether dying or killed were subjected to a post mortem examination. Major organs were weighed and a wide range of tissues preserved for histopathological examination.

Result : 2/10 females in the highest dose group died and one male at this dose level was sacrificed in extremis.
Clinical signs, when observed, were related to the degree of skin irritation that occurred during the study. There were signs of severe dermal irritation at the treated site consisting of scaling, scabbing and eschar formation. This severe irritation was seen in all rabbits treated with the test material. Although only minimal irritation occurred after the first exposure to the test material the severity increased as treatment progressed. In all treatment groups hyperirritability and hair loss was observed. In the highest dose group hind limb paresis and decreased motor activity were also observed. This was attributed to the degree of skin irritation since the affected skin became cracked with dense scabbing. The skin in these areas became tight and unpliable resulting in painful movement for the animals.
Body weight gains were lower than controls for the two highest dose groups. The low dose group had reduced body weight gains only in the third week of the study.
The following clinical chemical and hematological differences with controls were also observed:

	2 g/kg	0.67 g/kg	0.2 g/kg
SGOT	increased	-	-
Globulin	increased	-	-
Potassium	increased	-	-
Albumin/globulin ratio	decreased	-	-
Albumin	decreased	-	-
Alkaline phosphatase	decreased	decreased	-
Glucose	increased	increased	-
WBC	increased	increased	-
RBC	decreased	-	-
Hemoglobin	decreased	-	decreased
Hematocrit	decreased	-	-
MCHC	decreased	decreased	decreased
Differential WBC			
Mature Neutrophils	increased	-	-
Lymphocytes	decreased	-	-
Basophils	decreased	-	-

There were no significant differences in either male or female organ or organ to body weight ratios in any dose group when compared to the untreated controls. However when the data from both sexes were combined and these were compared to control values significant differences were found. These were as follows:

	Dose (g/kg)		
	2	**0.67**	**0.2**
Brain (absolute wt.)	low	low	-
Brain (relative wt.)	high	high	-
Liver (absolute wt.)	low	low	low
Liver (relative wt.)	high	-	-
Kidney (absolute wt.)	low	low	low
Kidney (relative wt.)	high	-	-
Heart (absolute wt.)	-	low	-
Heart (relative wt.)	high	-	-
Adrenals (absolute wt.)	-	-	-
Adrenals (relative wt.)	high	-	-

At gross necropsy skin changes were observed at all dose levels. These included thickened, scaly, flaky, cracked and crusty skin at the application site. Essentially all of the treated rabbits had enlarged prefemoral lymph nodes, some red in color. Several of the high dose rabbits also had enlarged axillary and inguinal lymph nodes. Stomach ulceration and thickened mucosa in some rabbits and small testes and ovaries in a few of the high and mid-dose animals was attributed to the stress of test article application or collar placement.

Histopathological examination revealed epidermal hyperplasia, hyperkeratosis, parakeratosis, dermatitis, necrosis and ulceration at the application site. The incidence was not dose-related but the severity was. There was a treatment-related enlargement of prefemoral lymph nodes and were due to a proliferation of lymphocytic and reticuloendothelial cells. Testicular tubular epithelial degeneration was observed in all study groups but there was a higher incidence in the groups exposed to the test material. This change together with giant cell formation was only observed in males in the high dose group.

Test substance : SAMPLE LF-7765 RI was described as a pink liquid with a viscosity similar to that of gasoline.

Reliability : (1) valid without restriction
Although it is not clear whether the study was carried out according to Good Laboratory Practices, it was subjected to a quality assurance assessment and appears to have been conducted using appropriate methods and to have been fully reported.

(26)

Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 24 hours
Frequency of treatm. : Daily for 5 days, two days rest and daily for further 5 days
Doses : 1, 3 & 10 ml/kg
Control group : Yes, concurrent no treatment
Year : 1979
GLP : Yes
Test substance : Diesel fuel, sample API 78-4 (predominantly saturates) See section 1.1.1.

Method : Undiluted test material was applied, using a four-inch square gauze, to the shorn skin of groups of 4 male and 4 female adult New Zealand White rabbits. Dose groups were 1, 3 and 10 ml/kg. An untreated group of 4 males and 4 females served as controls.
The applied gauze was covered with an occlusive dressing and left in place for 24 hours. After 24 hours it was removed and a fresh dose applied. This dosing regime was continued for 5 consecutive days, followed by two days rest and then with 5 consecutive days of further dosing.
Animals were observed for mortality, local reactions and behavioral changes. Initial and final body weights were recorded. Any animals that died during the study as well as those killed at termination were subject to necropsy. All significant gross pathological alterations were recorded.
Skin from the test site, liver, kidney, spleen and urinary bladder were submitted for histopathologic examination.

Remark : This study only demonstrated the irritant nature of the test material when applied repeatedly under occlusion to the skin. Any other changes were probably secondary to the severe skin irritation that occurred.

Result : Mortality and weight change in the respective groups was as follows:

Dose group (ml/kg/day)	Weight change (kg)	Mortality (%)
0	0.2 gain	0
1	0.08 gain	0
3	0.2 loss	25
10	0.7 loss	87.5

The most significant daily observation recorded at all three dose levels was the progressive deterioration of the skin at the treated site. It became thickened and necrotic and the animals were distressed by the treatment.

At gross post mortem, the 10 ml/kg animals were anorexic and had severe skin lesions. Treatment-related lesions were seen in animals at all dose groups.
The only significant histological findings were those associated with the severe skin lesions.

Reliability : (1) valid without restriction

(6)

Species : Rabbit
Strain : New Zealand white
Route of admin. : Dermal

Result : Four samples of distillate fuels have been tested in two week repeat dose toxicity studies. The results are summarized in the following table and, for completeness, these include the data from the preceding robust summary.

Sample	Dose (ml/kg/day)	Growth rate/ mortality	API Report No.
Home heating oils			
78-3	2.5	no effect)	
	4	no effect)	27-32773
	10	8/8 died, weight loss)	
78-2	1	no effect)	

	2.5	1/8 died)	27-32771
	10	6/8 died, weight loss)	
78-4	1	no effect)	
	3	2/8 died, weight loss)	27-32068
	10	7/8 died, weight loss)	

In all the studies, skin irritation was severe at all dose levels.

Diesel fuel			
79-6	4 ml/kg/day	no effect)	27-32817
	8 ml/kg/day	67% mortality)	

(4) (5) (6) (7)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. Typhimurium Strain TA 98
Test concentration : 1 to 60 µl
Metabolic activation : With and without
Result : Positive
Method : Modified Ames Assay
Year : 1991
GLP : No data
Test substance : 3 Samples of diesel fuel (See section 1.1.1. sample Nos. 22, 23 & 24)

Method : A modified Salmonella mutagenicity assay was performed at the Mobil Environmental and Health Science Laboratory. The technique that was used has been described elsewhere (Blackburn et al 1984 & 1986).

The middle distillate samples (2 ml) were dissolved in cyclohexane and the solution was then extracted with DMSO (10 ml). These extracts were tested in Salmonella typhimurium strain TA98.
The concentrations of DMSO extract used were: 60, 50, 40, 30, 20 15, 10 and 5 µl/60 µl. Extra concentrations were used for some assays.
Positive controls were 2.0 µg 2-aminoanthracene, 10.0 µg benzo(a)pyrene and 25 µg 2-nitrofluorene in 50 µl DMSO per bacterial plate.
Metabolic activation was accomplished by using an eight- fold higher concentration of the liver S9 fraction obtained from Arachlor-induced Syrian Hamsters rather than rats.
NADP cofactor was also increased from the normal 4 to 8 mM.

Result : A mutagenicity index (MI) was calculated which represented the slope of the dose response curve for each of the samples.
Previous studies have established that materials with an MI of less than or equal to 1.0 have not been associated with a tumorigenic response in skin painting bioassays, whereas those with MIs greater than 1.0 have been associated with a tumorigenic response.
The MIs for the middle distillate samples were:

Sample	**Mutagenicity Index**
22	1.7
23	3.9
24	2.0

The authors of the report concluded that the three samples of diesel fuel were mutagenic.

(10) (11) (15)

Type : Ames test

Result : Diesel fuel was evaluated in plate and suspension assays using S.typhimurium strains TA 1535, 1537, 1538, 98 and 100 and in yeast S. cerevisiae D4.
The assays were conducted without and with rat liver S9 activation.
The diesel fuel was negative in these assays.

(1)

Type : Mouse lymphoma assay
System of testing : Forward mutation assay using cell line L5178Y TK+/-
Test concentration : 0.125 to 0.5 µl/ml without activation and 0.064 to 0.5 µl/ml with activation.
Metabolic activation : With and without
Result : Negative
Year : 1978
GLP : No data
Test substance : No.2-DA (See section 1.1.1.)

Method : The test material was dissolved in ethanol for this assay.
Two positive control substances were used viz Ethyl methane sulphonate (EMS) at a concentration of 0.5 µl/ml and Dimethylnitrosamine (DMN) at a concentration 5.0 µl/ml.
Doses of test material used in the assay were based on the results of a cytotoxicity study carried out prior to the mutagenicity assay.

For the mutation assay the lymphoma cells were exposed for 4 hours to test material at dose levels ranging from 0.125 to 0.50 µl/ml without activation and 0.064 to 0.5 µl/ml with S-9 activation. After exposure to the test material, the cells were allowed to recover for 3 days. Mutants were detected by cloning the cells in the selection medium for 10 days.
Surviving cell populations were determined by plating.
A mutant frequency was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR.

A compound is considered mutagenic if:

a. A dose response relationship is observed over three of the four dose levels used
b. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control.
c. The solvent control data are within the normal range of the spontaneous background for the TK locus.

Result : The mutant frequencies and percentage relative growth are summarized in the following table.

Non-activation Group	**Mutant frequency**	**% Relative growth**
Solvent control	0.0231	100
Negative control	0.2143	126.1
EMS control	0.9758	37
Test material		
0.125 (µl/ml)	0.0658	59.0
0.188 (µl/ml)	0.085	44.5
0.25 (µl/ml)	0.3469	12.3

0.375 (µl/ml)	0.1264	17.3
0.5 (µl/ml)	0.0396	9.3
With activation		
Solvent control	0.0204	100
Negative control	0.0321	92.9
DMN control	0.9365	50.3
Test material		
0.064 (µl/ml)	0.0105	93.7
0.125 (µl/ml)	0.0129	98.5
0.188 (µl/ml)	-	22.7
0.25 (µl/ml)	0.0870	5.7
0.375 (µl/ml)	0.0183	2.1
0.5 (µl/ml)	0.1235	2.0

(1)

Type : Mouse lymphoma assay
Test substance : Sample API 78-4. See section 1.1.1.

Result : Home heating oil (API Sample 78-4, containing 50% cracked stock) was positive with and without S9 activation. (Ref API 27-30140)

(2)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Rat
Sex : Male
Strain : No data
Route of admin. : i.p.
Exposure period : Up to 48 hours and 5 days
Doses : 0.6, 2.0 & 6.0 cc/kg
Year : 1978
GLP : No data
Test substance : No.2-DA (See section 1.1.1.)

Method : Acute and subchronic studies were carried out. The design of the two studies is summarized in the following table.
In the acute study a single intraperitoneal dose was given to the animals which were then sacrificed at the time intervals shown. Two hours prior to being killed, colchicine was given as a single dose (4 mg/kg) to arrest dividing cells in metaphase.
Acute study

Treatment	**No. of animals killed hrs after dosing**		
	6	**24**	**48**
0.6 cc/kg	5	5	5
2.0 cc/kg	5	5	5
6.0 cc/kg	5	5	5
TEM positive control	5	5	5
Solvent control	5	5	5

In the subchronic study a group of 5 males at each dose level (same doses as for acute study) was given a single intraperitoneal dose of test material once every 24 hours for 5 days. Animals in this study were killed 6 hours

after the last dose was given. Cells were arrested in metaphase by the use of colchicine at the same level as that used in the acute study.

The positive control material was triethylene melamine (TEM), used at a dose of 0.5 mg/kg. The solvent control was 0.85% aqueous saline.

Bone marrow was aspirated from the canals of the femurs and tibias of the lower limbs. The cells from the bone marrow plug were washed, fixed and spread on slides and stained for examination. 50 spreads were located for each animal and when of suitable quality, the chromosomes were counted and evaluated for abnormalities.

Result : Similar aberrations were observed throughout the treatment and negative control groups, the only difference being the frequency of aberration.
The results of the evaluation are summarized in the following table.

Group	Time (hrs)	No of cells	Cells with 1 or more aberrations	No. animals without aberrations*	MI**
Saline	6	53	0 (0%)	2 (2)	0.7
	24	225	6 (2.6%)	2 (5)	3.2
	48	200	1 (0.5%)	3 (4)	3.6
	5 doses	250	2 (0.8%)	4 (5)	10.6
TEM	24	144	68 (47.2%)	0 (5)	1.4
Diesel 0.6 ml/kg	6	189	5 (2.6%)	0 (5)	5.9
	24	48	1 (2.1%)	4 (5)	1.7
	48	186	1 (0.5%)	4 (5)	2.3
	5 doses	250	4 (1.6%)	2 (5)	3.6
Diesel 2.0 ml/kg	6	228	8 (3.5%)	1 (5)	6.3
	24	86	5 (5.8%)	2 (5)	1.7
	48	216	2 (0.9%)	3 (5)	4.5
	5 doses	250	1 (0.4%)	4 (5)	7.4
Diesel 6 ml/kg	6	100	1 (1.0%)	1 (2)	4.0
	24	227	5 (2.2%)	2 (5)	4.7
	48	93	5 (5.4%)	2 (4)	3.8
	5 doses	200	10 (5.0%)	0 (4)	6.0

* ( ) Number in parenthesis is No. of animals examined.
** Mitotic Index

For each dose group, the total number of cells with aberrations were combined and the mean % aberrations was estimated. These data formed the basis for the report's conclusion, that at a dose level of 2 and 6 ml/kg diesel fuel was clastogenic.

Group	Total No. cells with aberrations	Mean % aberrations
Control	9	1.0
TEM	68	47.2
Diesel 0.6 ml/kg	11	1.7
Diesel 2.0 ml/kg	16	2.7
Diesel 6.0 ml/kg	21	3.4

Reliability : (2) valid with restrictions

(1)

Type : Dominant lethal assay
Species : Mouse
Sex : Male
Strain : CD-1
Route of admin. : Inhalation
Exposure period : 6 hours / day, 5 days / week for 8 weeks
Doses : 100 & 400 ppm
Result : Negative
Year : 1980
GLP : No data
Test substance : No. 2-DA (See section 1.1.1.)

Method : Groups of 12 male mice were exposed by inhalation to diesel fuel at airborne concentrations of 100 and 400 ppm. Exposures were for 6 hours a day, 5 days each week for 8 weeks (40 doses). A group of 12 male mice served as negative controls and were placed in the inhalation chambers but not exposed to diesel fuel. On day 40 of the dosing schedule a positive control group of 12 male mice were each given an acute intraperitoneal dose (0.3 mg/kg) of triethylenemelamine (TEM). Following completion of the exposure to test material the males were sequentially mated to two females per week for 2 weeks. After mating, the females were separated from the males and housed separately until killed. At the end of each 5 day mating period, the males were rested for 2 days and then mated with 2 new females.
14 days from the middle of the mating week, the females were killed and necropsied. At necropsy the uteri were examined and the number of living and dead implantations counted if present. Based on the data obtained at necropsy, the following parameters were calculated:
fertility index
total number of implantations
dead implantations
proportion of females with one or more dead implantations
proportion of females with two or more dead implantations
ratio of dead/total implants
ratio of living implants/total implants.

Result : The results were as follows:

	Dose Group			
Parameter	100 ppm	400 ppm	Negative control	Positive control
Fertility index				
Week 1	0.727	0.750	0.708	0.458
Week 2	0.955	0.792	0.833	0.792
Implants/female				
Week 1	11.06	10.72	11.18	8.36
Week 2	10.81	11.68	10.95	7.42
Resorptions/female				
Week 1	.375	.5	.77	4.73
Week 2	.71	.32	.55	4.9
Proportion of females with 1 or more dead implants				
Week 1	.32	.39	.41	1.0
Week 2	.52	.26	.55	1.0

Proportion of females with 2 or more dead implants				
Week 1	.06	.11	.24	1.0
Week 2	.14	.05	0	.95
Dead implants/Total implants				
Week 1	.03	.04	.07	.57
Week 2	.07	.03	.05	.66
Living implants/pregnant female				
Week 1	10.7	10.2	10.4	3.6
Week 2	10.1	11.4	10.4	2.5

Conclusion : The sensitivity of the assay was confirmed by the results with the positive control. The test material did not cause any significant pre- or post implantation losses when compared to the negative control.

(8)

5.7 CARCINOGENICITY

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Frequency of treatm. : Twice weekly for lifetime
Doses : 50 microliters
Result : Positive
Control group : Yes
Year : 1985
GLP : No data
Test substance : Diesel fuel LF-7765 RI
SAMPLE LF-7765 RI was described as a pink liquid with a viscosity similar to that of gasoline.

Method : 50 microliters of undiluted test material was applied to the shorn dorsal skin of a group of 50 male mice twice weekly for the lifetime of the animals. The applied dose was not spread mechanically or covered, but was allowed to spread of its own accord.
A group of 50 male mice served as sham-treated controls.
Animals were observed twice daily on weekdays and daily on weekends throughout the study. Any mouse considered not likely to survive until the next observation time was sacrificed and necropsied.
Body weights were recorded prior to initiation of the study, then weekly for the first four weeks, biweekly for the following 8 weeks and monthly thereafter. Animals with one or more skin masses were excluded from the body weight database to preclude skewing of the data. All mice were subjected to an extensive necropsy and a wide range of tissues were fixed for subsequent histopathological examination.

Result : Five mice died during the first nine weeks of the study. None of the deaths were considered to be treatment-related and were excluded from any subsequent statistical analysis.
The mean lifespan of the treated mice was 78.1 ± 24.2 weeks which was significantly shorter than 91.8 ± 82.5 weeks for the controls. When the data were assessed in 12 week intervals, the mortality rate of the treated mice was greater than the controls. There was a significant increase (9 vs. 0) in the incidence of malignant skin tumors (squamous cell carcinoma or fibrosarcoma) in the treated mice compared to the controls.
The mean time to appearance of histologically confirmed tumors was 94.4 ± 9.4 weeks.
The body weights and body weight gains of the treated mice were greater

than those for the controls. Unexplainably, by the end of the first year, the treated rats were on average 3 g heavier than the controls (33 vs. 3 g) and this difference remained throughout the study, the reason for which is not clear.
Seven of the 46 treated mice developed skin masses compared to zero out of 49 in the control group. The first of the masses was recorded during the 84th week. Other lesions of the treated skin included sloughing of the skin and lesions resembling infection both of which were seen more frequently in the treated animals. Increased motor activity lasting for one to two hours was a consistent observation in the treated mice but only seen in one of the sham-treated controls. Four treated mice were observed with rectal prolapse but this was not seen in the control animals. Some age-related changes were observed in both treated and control animals but hair loss, distended abdomens, abdominal masses and distended penises was observed more frequently in the treated animals than in the controls.
At necropsy none of the control mice had skin masses while masses were observed in seven of the treated mice.
Lesions observed in both groups but more frequently in the treated animals are shown below:

	Treated	**Control**
Final group size	46	49
Sloughing skin at the application site	41	1
Lesions resembling infection of the skin	18	2
Inguinal lymph node enlargement	29	16
Enlargement of the spleen	10	5
Distension of urinary bladder	8	1
Presence of calculi in the urinary bladder	4	0
Masses/nodules of the kidneys	5	0

All other observations at necropsy occurred in both treated and control animals at a similar incidence.

The only tissues that were processed for histopathology were the treated skin from all mice and all tissues considered to be outside normal limits at necropsy.
With the exception of those listed below, all lesions observed during microscopic examination were considered to be spontaneous and common for ageing mice of the strain.

	Treated	**Control**
Final group size	46	49
ORGAN/LESION		
Skin		
Squamous cell carcinoma	8/46	0/49
Fibrosarcoma	1/46	0/49
Acanthosis	33/46	1/49
Hyperplasia	1/46	0/49
Hyperkeratosis	2/46	0/49
Dermis - fibrosis/fibroplasias	26/46	0/49
Necrosis	8/46	1/49
Inflammation	3/46	0/49
Ulceration	1/46	0/49
Epidermal inclusion cyst	1/46	0/49
Accumulation of pigment	6/46	0/49
Dermatitis	1/46	0/49
Non-treated skin		
Squamous cell carcinoma	1/46	0/49

Liver*		
Hepatocellular carcinoma	22/29	25/36
Hepatocellular adenoma	4/29	4/36
Lungs*		
Bronchogenic carcinoma	0/7	2/17
Alveologenic carcinoma	1/7	1/17
Alveologenic adenoma	2/7	1/17
Hematopoietic lymphoreticular system*		
Malignant lymphoma	0	1/35
Pancreas+		
Islet cell adenoma	0	1/1

* Incidence of lesions in these organs based on number exhibiting gross abnormalities at necropsy.

Test substance : SAMPLE LF-7765 RI was described as a pink liquid with a viscosity similar to that of gasoline.

(27)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : Days 6 through 15 of gestation
Doses : 100 and 400 ppm
Control group : Yes
NOAEL maternal tox. : 401.5 ppm
NOAEL teratogen. : 401.5 ppm
Year : 1979
GLP : No data
Test substance : No.2-DA (See section 1.1.1.)

Method : Groups of 10 presumed-pregnant female rats (224-230 g) were exposed in chambers to diesel fuel at nominal atmospheric concentrations of 100 and 400 ppm. A further group of presumed-pregnant females served as controls. Exposures were for 6 hours each day from day 6 through day 15 of gestation.
Food and water were provided ad libitum except during the exposure periods.
The rats were weighed on days 0, 6, 15 and 20 of gestation.
Food intakes were assessed for the periods days 0-6, 7-15 and 16-20.
There were daily observations for general appearance, behavior and condition. On day 20 of gestation the rats were killed and the visceral and thoracic organs were examined. The uterus was removed and opened and a record made of the number of implantation sites and their placement in the uterine horns, the number of fetuses (alive and dead), and the number of resorption sites.
Fetuses were removed and weighed and were examined externally for abnormalities. One third of the fetuses were fixed for subsequent examination for changes in the soft tissues of the head, thorax and visceral organs. The remaining fetuses were examined for skeletal abnormalities after staining with Alizarin Red S. The uterus and ovaries were preserved in fixative for possible future examination.

Statistical analysis
Analysis of the data was performed using the litter as a basic sampling unit. Dunnet's t-test was used to determine statistical significance ($p<0.05$) for differences between means with near normal distributions (body weights, food consumption of dams, mean pup weight based on litter averages). Ratios (nidation index and live fetuses/implantation sites ratio) were analyzed with a 2 x 2 contingency table with Yate's correction. A Wilcoxon Rank Sum was used for discontinuous parameters (e.g. number of abnormal fetuses within a litter).

Result : After analysis of samples collected during the exposure the mean chamber concentrations of diesel fuel were found to be 0, 101.8 (± 2.4) and 401.5 (± 15.39) ppm.
There were no deaths during the study and all animals were normal in appearance throughout. At necropsy two 400 ppm animals had dark mottled lungs and one 100 ppm animal had two pups with a common placenta. Neither of these observations are considered to be treatment-related.
Body weights were not affected by exposure to the test material. Food consumption of treated and control animals was similar at the time periods examined except for the 400 ppm group which had a reduced food intake during the gestation period, days 7-15 only.
No treatment-related changes were observed on observation of the uterine contents.

There was no difference in sex ratios attributable to exposure to test material. The actual numbers were:

Exposure	Males	Females
0 ppm	29	32
101.8 ppm	35	37
401.5 ppm	35	25

No treatment-related differences were found in any of the following parameters:
Nidation index (females with implantations/bred)
Females dying prior to cesarean section
Live litters
Implantation sites (for each uterine horn)
Resorptions
Litters with resorptions
Dead fetuses
Litters with dead fetuses
Live fetuses/implantation site
Mean live litter size
Average fetal weight

Examination of the offspring at delivery revealed no visible abnormalities except for subcutaneous hematomas which occurred as follows:

0 ppm group	1
101.8 ppm group	4
401.5 ppm group	4

There were no abnormalities found in the soft tissues of the fetuses after examination of the Bouin's fixed specimens.

Some changes were observed during the skeletal examination of the stained fetuses. The "unusual changes" shown in the following table consisted of retarded bone ossification and were not malformations as

such. It was noted in the report that such changes, although not normal, were frequently observed in 20 day old fetuses of the strain and source of rat used in the study. The report concluded that neither the frequency nor the character of the changes indicated an adverse effect on fetal growth and development or a teratogenic potential.

Exposure (ppm)	Fetuses examined	Fetuses normal	Fetuses with commonly found changes	Fetuses with unusual skeletal variations
0	127 (16)	70 (16)	50 (14)	7 (3)
101.8	155 (19)	68 (16)	70 (18)	17 (8)
401.5	128 (16)	66 (16)	62 (15)	0 (0)

Figures in parenthesis indicate No. of litters

Reliability : (2) valid with restrictions
The study was subjected to a Quality Assurance inspection but there is no information regarding compliance with GLP requirements. Nevertheless the study was well conducted and reported and is considered reliable.

(3)

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Initiation/promotion assay
Species : Mouse
Sex : Male
Strain : CD-1
Route of admin. : Dermal
No. of animals : 30
Vehicle : Undiluted
Control group : Yes
Year : 1993
GLP : Yes
Test substance : Diesel fuel, DGMK Sample No. 22 (See section 1.1.1.)

Method : A sample of a diesel fuel was investigated in this assay.

50 µl of test material was applied undiluted to the shorn dorsal skin of 30 male CD-1 mice for 5 consecutive days of the first week of the initiating period.

Promoter: TPA (12-0-tetradecanoylphorbol-13-acetate),applied (5 µg/animal, dissolved in 50 µl acetone) twice a week from week 4 to week 28.

Initiator DMBA (7,12-dimethylbenz(a)anthracene) applied once (50 µg/animal, dissolved in 50 µl acetone) on the first day of the initiating period.

The treatment groups were as follows:

Group	Treatment
0	Acetone/TPA
1 negative control	DMBA/Acetone
2 negative control	DMBA/TPA
Test for initiating activity	
14	Diesel fuel/TPA
Test for promoting activity	
15	Diesel fuel/DMBA

The treatment regimes were:
For assessing tumor initiating potential, the test material was applied to the mice for 5 consecutive days during the first week of the initiating period.
TPA was applied twice a week from week 4 to 28.
Body weights were determined once weekly and once each week a detailed examination of the skin was performed.
At the end of the study, the animals were assessed grossly followed by histopathology of the skin and all macroscopic lesions.

Assessment of promoting activity
The animals were initiated with DMBA once on the first day of the administration. From week 4 to 28 the test material was applied to the mice twice a week.

During weeks 2 and 3 of the study, i.e. between initiation and promotion, the animals were untreated to allow a regression of possible skin alteration.

Result **:** The diesel fuel sample did not cause any body weight changes compared to controls.
Survival of the animals was also unaffected by exposure to the diesel fuel sample.
During the initiation phase, the diesel fuel sample caused slight skin irritation consisting of reddening, scale formation, and/or erosions in four of the 30 animals.
During the two week recovery period between the initiation and promotion phase the skin changes were found to be reversible.

Similar skin changes also occurred during the promotion phase.

The number of animals with neoplastic findings in the treated skin at the end of the study was follows:

Test group	**treatment**	**No. of animals with squamous cell papilloma**
0	Acetone/TPA	0
1	DMBA/Acetone	0
2	DMBA/TPA	30
14	Diesel fuel/TPA	0
15	DMBA/Diesel fuel	1

The authors concluded that the diesel fuel sample may be a promoter.

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